Microscopical Examination of The Human Brain

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THE MICROSCOPICAL EXAMINATION

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METHODS.

WITH APPENDIX OF METHODS FOR THE PREPARATION OF THE BRAIN FOR MUSEUM PURPOSES.

BY

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PREFACE.

THIS compendium of methods for the preparation of the human brain for microscopical examination and museum purposes is published in the hope that it may be of some service to those who are devoting attention to cerebral pathology. The number of workers in this particular field has greatly increased of late years; especially in asylums for the insane is there occasion to observe a quickening of interest in questions relating to the morbid histology of the brain.

In the present work the brain alone is considered; it is proposed to deal with the methods employed in the microscopical examination of the spinal cord and nerves on a future occasion.

For many important references my acknowledgments are due to the excellent "Microtomist's Vade-Mecum" of Bolles Lee. My friend and colleague, Dr. Maurice Craig, has my best thanks for the Index—almost entirely compiled by him—and for much assistance in the revision of the proofs.

E. G.

December, 1893.

MICROSCOPICAL EXAMINATION OF THE BRAIN.

I.—FRESH METHODS.

Freezing Method (Bevan Lewis)—To show structure of **Cerebral and Cerebellar Cortex.**—Remove a piece of brain (cortex and white matter) about $1\frac{1}{2}$ cm. by 1 cm.; height, 5–8 mm. It should be as fresh as possible. It is important to retain the pia mater, in order that morbid conditions in and beneath it may be studied. If the piece of brain removed consist of the adjacent portions of two gyri, the fold of pia dipping into the intervening sulcus will be secured with a section, and probably some of the surface (exposed) pia also. Place the piece on an ether-freezing microtome [Lewis, Cathcart, or other] with pia nearest to knife; thus a better chance of including the membrane in the section is afforded. Freeze to the level of the section-plate. The temperature of the room should not be above 60 degrees, Fahr. The knife used should have both surfaces hollowed, especially that uppermost in cutting. Placed on a flat surface, the back and edge should be on the same plane. Size, 6 inches by $\frac{3}{4}$ inch. [Made by Young, or Gardner, Sur-

gical Instrument Makers, Edinburgh.] Before use, the upper surface of the knife should be prepared so that water will lie upon it uniformly up to the edge as follows :—Cover the surface with ether, and plunge the blade, so covered, into water. Repeat this two or three times. The same purpose is effected by passing the blade a few times through the white matter of the brain. The knife is now ready for use. It may be said here that it is quite possible to obtain good sections with a razor, or even plane-iron. The knife described, however, is a convenient length, and comes in useful for cutting in wax also. Wipe the under surface on a towel placed over the knee. Remove the unfrozen tissue by a sweep of the knife. Cut sections by a pushing movement. The best are obtained at the junction of the frozen and unfrozen tissues. They should be very delicate, and float on the blade. Float them on to the surface of water kept at hand in a suitable vessel. The kind used in Koch's plate-culture process is useful. Two such glass vessels, filled with water, should be at hand. Take up the sections on a slide as soon as possible, and float up each section with osmic acid, $\frac{1}{4}$ per cent. sol., drawing the fluid completely over it also.

The osmic acid is allowed to act about $\frac{1}{2}$–1 min.; its action is fixative. Wash the section in water, to remove excess of the osmium, and then stain, on the slide (for very delicate and friable sections),* or in a porcelain dish, using filtered aqueous solution of English anilin blue-black ($\frac{1}{4}$ per cent.). This is allowed to act about one hour. Wash well in water (*distilled* finally). Take up on slide, and allow the section to dry

* Taking care that the stain gets beneath the section.

thoroughly on it, under cover. Drying hastened by keeping the slide on the slant; and still more, by placing it beneath a bell-jar with a capsule containing strong sulphuric acid. When dry, apply a drop of canada balsam; cover. By this method nerve-cells—and their processes, in considerable extent—and connective-tissue-cells and nuclei are stained blue-black. The stain is especially adapted for nerve-cells. The neuroglia-matrix appears of a grey-blue tint (very faint, unless the specimen is diseased).

The method is a very important one for the study of healthy cerebral cortex, and for the demonstration of changes in nerve and connective-tissue-cell, and vascular changes in the morbid brains of alcoholism, general paralysis of the insane, and other varieties of insanity.

By staining a section with picro-carmine as well as anilin, connective-tissue and vascular structures are better shown than by the ordinary method. Stain on the slide with picro-carmine, 10–20 minutes. Drain off, and stain with anilin blue-black, $\frac{1}{4}$ per cent. aq. sol. This acts rapidly after picro-carmine has been used—about 10–15 minutes required. Proceed as above.

Other anilins do not give equally good results with the blue-black in staining fresh cortex; unlike the latter they stain diffusely, not selectively. Hence it is necessary to remove excess of dye. Alcohol is, of course, prohibited for this purpose. Very dilute acetic acid may be employed; it removes a considerable amount of colour from the ground substance, but at the expense of the nerve-cells. The writer has obtained fairly good results as regards these elements, with dilute aq. sol. gentian, or methyl-violet, or dilute aq.

sol. Lœffler's alkaline methylene-blue. Connective-tissue nuclei and those of vessel-walls are brought out much better by these dyes than by anilin blue-black—as might have been expected from experience with hardened tissues. They are therefore useful when it is desired to show abnormal vascularity of the cortex (as in the case of general paralysis).

The fresh *unstained* section may be examined with advantage. Colloid bodies, dilated and congested vessels, perivascular exudates, miliary apoplexies, &c., show well. The first named (colloid bodies) are unaffected by anilin blue-black, but stained by haematoxylin or osmic acid. By reflected light nodules of miliary sclerosis can be seen as minute opalescent spots.

Congested capillaries are well shown in thin sections mounted in physiological salt solution.

Fresh Sections of Central Medulla or Basal Ganglia (Lewis).—Cut on freezing microtome, as above, and take up sections on slide. They may now be examined under a low power; medullated nerve-fibres show well. If to be preserved, remove the water from the section as quickly as possible and float up on osmic acid, 1 per cent., which is drawn also over the surface. Expose to this reagent 5–10 minutes under cover. Wash in water. Medullated fibres are stained by the osmic acid. To stain the grey matter, place sections in picro-carmine a few minutes. Wash off excess of stain, and float up the section with glycerine for 5–10 minutes; it is thus freed from much water. Drain off this glycerine and mount in pure glycerine, Farrant's or Goadby's solution, cementing down the cover-glass.

The broken-down, pulpy tissue from foci of *softening* (blood-crystals, compound granule corpuscles, varicose

nerve-fibres, &c.), is best examined fresh, pressed out to a thin film between cover-slip and slide. The film may, if desired, be submitted to the action of dilute osmic acid ($\frac{1}{6}$ per cent. for 1–2 hours in the dark), to stain compound granules and nerve-fibres. Supplement the procedure by hardening methods. The corpora amylacea found about sclerotic foci in the white matter are well shown in similar preparations, or in fresh sections. Iodine stains them blue-grey or blue-violet.

Dissociation—Preparations to show the Ganglion Cells of Cortex (Lewis).—A piece of convolution, stripped of its pia, and of convenient size, is held between the thumb and second finger of the left hand, the left index guiding the razor-blade, which, saturated with spirit, is used to cut as delicate sections as possible, vertically to the surface of the cortex. Place the section on a glass slide and allow a few drops of Müller's fluid to fall on it from a pipette. Macerate in this a few moments. Then apply the cover, arranged to cover the film by one-half its diameter. Flatten out the section into a thin film by pressing on the cover-slip by a mounted needle. Rinse the slide in water to remove superfluous Müller, and place it in methylated spirit for 30–40 seconds. The cover can now be removed without tearing the film. Carefully wash the latter free from spirit by water from a camel-hair brush. It is then stained as it lies on the slide with anilin blue-black 1 per cent. aq. sol. When enough stained, lower the slide into water gently and wash the film carefully. Dry, and then apply a drop of chloroform to the specimen, immediately afterwards mounting in balsam (chloroform or benzole solution).

Dissociation—Preparations to show the Neuroglia (cells and ground substance) in the White Matter* (Boll).—Thin sections of brain-substance are thrown into a solution of osmic acid 0·1 per cent., and left for twenty-four hours. Wash in aq. dest., and transfer to concentrated sol. potass. acetate. Here they remain an unlimited time. The sections are then either teased or compressed strongly between cover and slide.

The nerve-fibres appear black, connect. tiss. cells and their processes unstained.

Same after Mierzejewski.—A portion of the white matter about 1 ccm. in bulk is placed in osmic acid, 1 : 300, for 12–24 hours. Slice off the hardened exterior, and tease out a piece from the interior in aq. dest. Stain with picro-carmine and examine in glycerine.

For the same purpose (teasing of neuroglia), *Gierke* recommends the following, as maceration fluids. Very weak sol. of chromic acid or its salts; amniotic fluid, iodized serum [*see* below], Ranvier's one-third alcohol. Most suitable of all is the liquid of Landois [*see* below]. Pieces are kept in it for 2–5 days; then for twenty-four hours in ammonia-carmine solution, diluted one-half with the same fluid. Tease in water; allow this to evaporate, and when quite dry mount in balsam.

Teasing—for the demonstration of nerve and glia cells—may also be practised after maceration in one of the following fluids.† Use a very small piece of

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* For showing the cells of neuroglia in teased out preparations, quite fresh material is needed. Human brains as ordinarily obtained are unsuitable; isolation of the glia cells in such is very difficult. The central nerve organs of ruminants are best adapted for these preparations.

† The spinal cord is more commonly used for teasing purposes.

tissue, say a cube 2–3 mm. (1.) *Dilute alcohol* ("one-third": alc. absol. 30, water 70). Macerate in this 1–2 weeks. (2.) *Osmic acid* sol., 0·1 per cent., twenty-four hours. After 7–14 days good preparations can also be obtained. (3.) *Müller's fluid* (pot. bichromate 2–2½ gram., sulphate of soda 1 gram., water 100 ccm.): dilute freely and use 2–4 days. Or (4) the Müller may be used for several days, until the tissue is rather firm; wash, place in abs. alcohol a day or two. Then for several weeks in following solution: glacial acetic acid 200 ccm., creasote 20 drops, water 800 ccm. If too soft after this treatment, place the tissue in pure pyroligneous acid a few days (Stilling's method). (5.) *Landois solution* (sat. sol. neutral chromate of ammonia 5 parts, sat. sol. phosphate of potash 5 parts, sat. sol. sulphate of soda 5 parts, dist. water 100 parts). Macerate in this 1–5 days. Stain twenty-four hours in ammonia-carmine diluted with one vol. of macerating fluid. (6.) *Iodized serum* (blood-serum 100 ccm., tinct. iodi. 1 ccm., carbolic acid 2 drops). (7.) *Salt solution* (0·75 per cent.). Tease in the last two forthwith or after brief use (few minutes to an hour).

For teasing a dissecting microscope or lens is useful, and a couple of mounted needles are requisite. Carry out the process in a little distilled water on the slide. The preparation may be stained by adding a little carmine or fuchsin to the macerating fluid used, or after teasing, by means of ammonia-carmine or aq. sol. anilin blue-black, or this combined with picro-carmine. In the latter case either allow the tissue to dry sufficiently to adhere to the slide before staining, or press a cover-slip upon it and run in the stain between cover and slide. When stained separate the

two, and remove excess of dye by blotting-paper. Preparations, freed from excess of stain, are mounted in glycerine, or—after drying—in balsam.

Vignal employs principally the following method in examining nerve and neuroglia cells in embryonic brain. A small piece of the tissue is placed in one-third alcohol for twenty-four hours; minute portions are then shaken up in about 12 ccm. dist. water in a tube. Picro-carminate of ammonia is then added (1 ccm. of a 1 per cent. sol.) for staining; the elements are allowed to settle. They are presently—when sufficiently stained—taken up by a pipette, and placed in about 12 ccm. dist. water in a fresh tube, to which is added 1 ccm. of osmic acid 1 per cent. The tube is kept closed, and in about twenty-four hours fixation is complete. Decant off the water and examine some of the deposit microscopically, mounting in carbolic acid sol. 1–1000.

For the examination of the smaller *cerebral blood vessels* and the adventitial lymph space, teased-out preparations of fresh brain, or of portions of brain which have been macerated 24–48 hours in a very dilute sol. potass. bichrom. (or other of the macerating fluids mentioned), are the most suitable. The fresh preparations may be treated for a short time with a weak sol. acetic acid (5 drops to 50 ccm. water) before staining. For staining, picro-carmine, hæmatoxylin, and most analin dyes are suitable, especially bismarck brown. Fresh preparations may be mounted in dist. water or salt solution. Löwenthal recommends that the cover should be sealed down by balsam or dammar as soon as its edge is dry; preparations then last for a long time. Preparations of

macerated tissue may also be stained by any of the dyes mentioned and mounted as above.

Many morbid conditions of cortical blood-vessels are well shown in fresh ether-frozen sections.

The blood-vessels coursing on the under surface of the pia, and the branches given off by them to the cortex, may be shown by the following procedure. The fresh brain may be taken, or a piece of cortex and pia which has been slightly macerated in a suitable fluid (see the macerating fluids given). Carefully strip off a small piece of pia; with it will be taken the blood-vessels referred to. Place the whole in some carmine stain. As good as any is Schweigger-Seidel's acid-carmine—ammonia-carmine slightly acidified by acetic acid. Wash out with HCl. sol. 0·5 per cent. [Much used by Boll in his investigations.] After staining examine in glycerine. Around the vessels can be seen sheaths, here loosely, there closely applied. [The vessels on under surface of the pia are referred to.] In the space between sheath and vessel-wall are lymph-corpuscles in varying number. A marked excess of leucocytes is easily appreciated. The investing membrane is the adventitial lymph-sheath. Boll states that the sheath is best seen after maceration in concentrated oxalic acid instead of the ordinary chrome-solutions.

Small strips of fresh pia may also be stained by some of the anilin dyes (bismarck-brown, methyl-green, &c.), by weak osmic acid ($\frac{1}{6}$–$\frac{1}{4}$ per cent., to show fatty changes in vessel-walls), and treated by weak acetic acid (to bring out the nuclei of the vessel-wall). The minute arteries and veins, and the capillaries of the pia are well shown in such preparations, which

are temporary. Mount, outer surface downwards, in normal salt-solution or distilled water.

If the subject of arterial degeneration is to be investigated, it is advisable to examine the fine vascular twigs passing to the basal ganglia and pons varolii from the vessels at the base of the brain. Draw them out by gentle traction upon the main trunk, snip off with fine curved scissors, and float up in water on a slide. Plugging of the vessels, degeneration of their walls, and aneurismal dilatations can be thus seen.

Large cerebral arteries may be hardened by the ordinary means, cut in gum, paraffin, or celloidin, and sections stained by the ordinary dyes.

The *dura mater* can be examined in the fresh state by cutting sections between two pieces of liver by razor, or in a sliding microtome; or cut on the freezing microtome. This is a useful way of examining pseudo-membranes. Mount in normal salt solution or distilled water. Sections can also be treated by dilute acetic or osmic acid, and stained by anilin and other dyes. As regards hardening and subsequent processes preparatory to examination, the membrane may be treated like other tissues. It is well to pin it out on a piece of cardboard whilst hardening.

It is sometimes necessary to examine for bacteria, as in cases of tubercle of membranes or of brain, purulent meningitis, or when foci of acute inflammation are found ("hæmorrhagic encephalitis"). Cover-slip preparations and sections of the tissue, hardened in alcohol, should be made, and examined for organisms as usual. Of exudates, cover-slip preparations can be prepared.

II.—INJECTION OF CEREBRAL BLOOD-VESSELS.

The two following injection-fluids may be recommended for ordinary use.

Carter's Carmine Injection.

Carmine (*pure*)	1 dr.
Strong sol. ammonia	2 fl. drs.
Glacial acetic acid	86 m.
Sol. of gelatine* (1–6 water) . . .	2 ozs.
Distilled water	1½ ozs.

Dissolve the carmine in the ammonia and water, filter after allowing to stand an hour. Add the acetic acid, and stir well. Add the gelatine solution, stirring thoroughly. The fluid ought to be slightly acid. The gelatine is softened before dissolving it by allowing it to stand (cut up) in a little distilled water, which is gradually absorbed. Some thymol or salicylic acid is added to the fluid before it sets, for preservative purposes.

Prussian-blue Injection.

Soluble Prussian-blue	2 grammes.
Best French gelatine (gold label)	7 grammes.
Distilled water	91 ccm.

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* Best French gelatine, gold label (Coignet's).

Dissolve the powder in half the water, rubbing it up well in a mortar. In the remaining water the gelatine, cut into small pieces, is allowed to swell up, this takes some hours. It is then dissolved in the water by heat. Add the blue solution, little by little, with much stirring. Filter through fine flannel. Vessels coming in contact with the solution should be rinsed out with slightly acidulated water (·2 per cent. HCl., Fearnley) beforehand. The blue solution may either be filtered before adding it to the gelatine, or—what is quicker and equally satisfactory—it is allowed to stand until undissolved particles have gone to the bottom, the decanted fluid is then employed.

Reliable Prussian-blue is supplied by Gruebler.

Should a yellow injection fluid be required *Hoyer's* **Lead Chromate** may be used. Make—

(*a*) A solution of gelatine in water (1 : 4). This should be clear.

(*b*) A cold saturated solution of potass-bichromate.

(*c*) A cold saturated solution of sugar of lead.

The solutions may be kept ready prepared. Add (*b*) to (*a*), mix well, warm almost to boiling, and gradually add (*c*), previously warmed. Equal volumes of the three solutions are employed. Proceed with injection as soon as the fluid is of suitable temperature.

Aqueous Sol. Anilin Blue-Black makes a very fine fluid owing to the solubility of the dye. It may be used as $\frac{1}{4}$–$\frac{1}{2}$ per cent. sol. (stronger if the tint is considered too pale) combined with gelatine 5 per cent.

Silver-Nitrate.—An injection of this substance has been recommended for the study of cerebral blood-vessels. As an aqueous solution, $\frac{1}{4}$ per cent., silver-nitrate has been long employed for injecting blood-vessels in various parts of the body. According to Valenti and d'Abundo, a fluid of similar strength—in any case, not exceeding 0·5 per cent.—forms the best injection for the study of the vessels in the brain of mammals. They remark that when stained gelatine is injected the vessels, especially in embryoes, are distorted from their normal position. The $AgNO_3$, by penetration into the walls, brings out the endothelial lining of the vessels. Further, it is stated that the perivascular lymphatic sheaths are shown in this way. The best after-treatment is said to be 20 minutes' exposure to direct light, followed by hardening in alcohol.

The writer has employed the injection upon the brains of freshly-killed adult rabbits. After exposure to light fresh sections were cut from one hemisphere, the other was hardened in alcohol. The fresh sections were examined at once. The nitrate of silver was found to have taken effect upon the vessels running from the pia into the cortex, but for a short distance only. No small vessels were mapped out. The sections from the hardened hemisphere showed no silver-reaction.

If silver-nitrate is to be used for injecting the cerebral blood-vessels the brain must be from an animal just dead, as fresh tissue is essential. It is best to kill the animal and inject at once through the aorta (in the case of a small animal), washing out the vessels with distilled water before using the silver-solution. Inject from a glass syringe.

Alferow recommends the silver-salts of organic acids (lactate, acetate, &c.), as giving better results than the nitrate. The lactate solution is as follows:—

Lactate of silver, 1 part.
Dist. water, 800 parts.
Conc. sol. lactic acid, a few drops (10–15).

The free acid, according to Alferow, decomposes precipitates formed by the action of the silver on chlorides and other substances in the tissues, leaving intact the albuminate only.

Taguschi's Cold Injection of Japanese Ink.—The success of this injection when employed on other organs renders it worthy of trial in the case of the brain; it has not yet been tried, as far as the writer is aware. Japanese ink is well rubbed up with water until a deep black fluid is obtained, which does not run when dropped on thin blotting paper nor form a grey ring around the drop. Inject with the ordinary instruments. The organ becomes quite black. Cut into small pieces and harden in any way suitable. Prepare sections as usual. The injection is very fine.

For the purpose of injection a *brass syringe* frequently answers well, especially a stomach-pump pattern of suitable size, which has the advantage that no detachment for refilling is necessary. An objection to the syringe is the difficulty of maintaining an equable flow with it. The piston should not be pushed but screwed down, to ensure as much steadiness of pressure as possible. Again, the gelatine tends to set in the syringe. To prevent this, dip the syringe from time to time in the hot water surrounding the bottle containing the injection-fluid; the latter should be rather overheated, to

allow for some cooling in the syringe. The instrument is provided with cannulae of different sizes, and a stop-cock removable from both syringe and cannula.

In removing a brain for injection the dura mater should be left attached to the inner margin of each hemisphere, and the lepto-meninges kept as intact as possible. Should there be much atheroma of the basal vessels the brain had better be discarded at once.

Place the organ in a basin of water sufficiently warm to keep fluid the circulating injection mass. The bottle to contain the latter is kept in another basin of warm water, placed alongside the first. An india-rubber tube passes from this bottle to one nozzle of the syringe. The other nozzle (the stomach-pump pattern is referred to) communicates, through the stop-cock, with the cannula. Proceed as follows:—Filter the injection-fluid into the bottle through flannel wrung out of hot water. Tie a cannula of suitable size into one of the internal carotid arteries, placing clips upon the basilar and other carotid. Push the stop-cock into the cannula and fill both with injection fluid by means of a pipette; turn the stop-cock. The syringe is then filled, air being expelled by holding the nozzle uppermost and forcing out some of the injection. Allow a few drops to fall into the stop-cock piece to fill it. Unite syringe and stop-cock, turn the latter, and inject slowly. One attempts to get as complete an injection as possible of the capillaries of the pia. It must, however, be admitted that the state of the pia, as regards injection, is no certain criterion of that of the subjacent cortex. Should an escape of fluid occur by some accidentally-injured surface-vessel this should be clipped if possible. In the brains of the chronic insane it is only too common to

get small scattered extravasations of the injected-fluid; but in healthier brains these are of course much rarer. The injection of one hemisphere—or as much as is desirable through the carotid—being accomplished, that of the other is performed, through its carotid, the vessel just used being clipped. Subsequently the basilar artery is injected, or a vertebral artery, the other, and the carotids being clipped. The amount injected varies according to the state of the vessels; sometimes 20 ozs. is enough, at other times—probably when the small cerebral arteries are diseased—30 ozs. does not appear excessive. When the process is completed the stop-cock is turned off, the syringe withdrawn, and the brain removed to cold water. Here the gelatine quickly sets. Subsequent treatment is commenced as soon as possible.

Fresh sections should be cut forthwith by the ether-freezing method. The carmine injection (either this or a yellow fluid should be employed in this case) forms an excellent contrast with the anilin blue-black of the nerve-structures. This method of examining injected brains is very instructive, and worthy of much more attention than it has received. As organs injected with carmine are best hardened in alcohol—which produces much shrinkage of brain—there is the greater reason for examining fresh in the present instance. With Prussian-blue and anilin blue-black, subsequent hardening in chrome-salts is quite suitable. Prussian-blue sections are best cleared in turpentine and mounted in turpentine-balsam, as turpentine preserves the colour, which tends to fade. Yellow injection masses also fade in time, whereas red ones are practically permanent.

More reliable than the syringe for the purpose of

injection is a continuous *air-pressure* apparatus,* the principle of which is that the fluid is driven in continuously by compressed air. The compressing agent is usually water. A large Wolff's bottle (*a*), with three orifices, is employed. One orifice is connected by tubing either with a water-tap or a large vessel holding water, and capable of being raised or lowered at will. A second orifice is connected with a mercury manometer, and the third with the nearest orifice of another Wolff's bottle (*b*), containing the injection-fluid, and provided with two orifices. The further one is connected by tubing to the cannula inserted into the blood-vessel used for injection. Bottle (*b*) and the brain are placed in a water-holding trough, the water in which is kept at a constant temperature by a Bunsen burner beneath, regulated by a thermo-regulator. Fill the tube from (*b*) with injection-fluid before attaching it to the cannula—already filled. The corks of the bottles should be of vulcanised india-rubber.

Air is driven from (*a*) by the water flowing into it; passing into (*b*), the air forces the injection from that vessel into the brain. The pressure exerted upon the air in (*a*) is indicated by the manometer, and is regulated by duly controlling the water flowing from the tap, or by raising and lowering the water-receptacle used. The pressure should commence at one-half inch mercury, and be gradually increased to about 4 inches.

Very successful injections of cerebral blood-vessels may be obtained in small animals (such as the rabbit) from the aorta. After tying the cannula into the aorta just above the heart, clipping the arteries to the upper

* For an improved form of injection apparatus, *see* Middlemass *Journal of Pathology and Bacteriology*, February, 1893.

limbs, and the upper end of the thoracic aorta, and cutting across the jugular vein on each side, the entire animal may be placed in warm water. Warm salt-solution is first injected for a few minutes. The carotids become tense, and the return flow can be seen issuing from the cut jugulars. Then use carmine-injection. When the ears, conjunctiva, gums, &c., of the animal are deep-pink [the operation should not be hurried] clips are placed on the aorta and jugulars, the injection-tube is detached, and cold water substituted for warm. The brain should be cut fresh. In this way a wealth of fine vessels is shown. Unstained sections should be compared with others, stained with anilin blue-black as usual.

Injection of Cerebral Lymphatics.

The puncture-method is the only one employed, and is far from satisfactory. By thrusting the point of a fine glass cannula into the substance of the brain, it is possible to inject some of the perivascular spaces. [These are not commonly regarded as lymphatics, but it is convenient to refer to them in this connection.] Proceeding after the method suggested by Boll, the cranium and dura mater are removed to as small an extent as possible, and a cold solution of Berlin-blue (or other cold injection-fluid) is injected slowly into the cerebral substance. In the course of this procedure an irregular extravasation first appears ; then the fluid spreads in the direction of least resistance—that is, it follows the blood-vessels, spreading between them and the brain-substance. The fluid further passes from the perivascular channels into the epicerebral space, and

sometimes a limited injection of the lymphatics of the pia mater is obtained. Obersteiner injected the perivascular spaces from the epicerebral space by thrusting a glass cannula through the pia (in the neighbourhood of a blood-vessel) a short distance into the brain-substance, and then raising the point until it came in contact with the pia; the fluid was then injected beneath the pia under a pressure of 20–30 mm. mercury. Obersteiner states that the cornu ammonis is a particularly suitable part for injection purposes.

Boll obtained an injection of the lymphatic meshwork in the pia by inserting the point of an injection-needle into the membrane. In this way also the adventitial lymph-space of cortical blood-vessels can be injected for a short distance, the fluid passing along the vessels from the pia.

Taguschi states that he has injected lymphatics "in the most different organs" by means of the cold injection of Japanese ink referred to above. The fine character of this fluid is valuable for the purpose. It may prove serviceable in the case of cerebral lymphatics.

III.—HARDENING METHODS.

Generalities on Hardening.—The tissue to be hardened should be as fresh as possible.

If desired to harden large pieces, incise them as deeply as possible in the less important regions, unless there are special reasons to the contrary. Separate the hemispheres at the corpus callosum, unless this is for some reason undesirable.

As a rule, pieces of moderate size only should be hardened. Use the fluid plentifully. Proportion of fluid to tissue about 1 : 20.

Remove only the dura mater at first. It is often recommended that the lepto-meninges should also be removed, at a later period, when hardening has made some progress. Practically it is quite sufficient to strip them off here and there, at the least important sites. A portion of brain from which sections are to be cut should have the pia attached if possible, in order that morbid conditions in and directly beneath the membrane may be observed.

Lay out organs in the hardening fluid upon cotton wool. They should not be placed one above the other.

To prevent decomposition, keep the preparation in a cool place, preferably an ice-safe. On the other hand, the hardening is quickened by a temperature higher than normal (about 30–35 degrees C.) Tissues

can thus be prepared for cutting often in less than one-half the time needed for hardening in the cold. But it is doubtful if the results are equal to those obtained by the latter method; many think not.

Harden in the dark when solutions of the chrome-salts are used, as light decomposes the latter in some way.

The minimum of shrinking is secured by passing from weaker to stronger solutions. Change before muddiness appears in the fluid.

Do not leave tissues in the solution used for hardening longer than is necessary for the completion of the process; place them in a very weak solution of the same reagent or in some preservative fluid. Brittleness of substance indicates too prolonged or too rapid hardening.

No water should come in contact with a piece of brain which is to be hardened. Place direct from the body into the hardening fluid.

Add a little camphor to the latter to prevent formation of moulds.

A statement by Honegger may be given here, to the effect that quite old, over-hardened brains may be rendered fit for cutting and staining (supposing no decomposition to have occurred during or before hardening) by placing them for several days in water kept near the boiling point and often changed.

To Harden Whole Brain.—Hamilton's Method is a good one. On removing the organ, care should be taken not to lacerate the brain tissue. Inject on removal, with Müller's fluid* through each carotid and one vertebral, tying the other. By connecting the

* *See* Teasing Methods for its composition.

injecting cannulae with the stop-cock of a tank (raised to the height of about 4 feet) containing the fluid, the injection may be continued for some days (7–14). Replenish the tank every day. The brain lies meantime in a suitable vessel, into which the injection passes, after percolating slowly through the organ. The first Müller's fluid is to be thrown away, but that passing subsequently may be collected in an overflow vessel and used again and again. When the injection is complete the brain is allowed to lie in the Müller till hardening is finished—a period of some 3 months (12 months and more not detrimental). Or, after 8–9 weeks, hardening may be hastened by cutting the organ into slices $\frac{1}{2}$–1 inch thick, and placing these in spirit for some days.

To Harden Cerebral Hemispheres.—(Betz's Method.)—Divide the cerebrum into two along the median line of the corpus callosum. Place each half into following solution:—Alcohol, 75–80 per cent., iodine sufficient to produce a light-brown colour. After a few hours remove the pia mater from the Sylvian fissure and corpus callosum, also the choroid plexus if possible. Keep the preparation lying in the iodine solution in a cool place; add fresh iodine as soon as the liquid changes colour. In 24–48 hours remove all the remaining pia; add one-half vol. of fresh iodine solution. Stuff all recesses and fissures with cotton wool to facilitate penetration. In 24–72 hours place the hemispheres in fresh solution of iodine in alcohol, 70 per cent.; here they remain 10–14 days. Transfer to pot. b chrom. sol. 4 per cent., and leave in this till sufficiently hard. If much brown deposit forms during the process, rinse with water, and change the solution.

When ripe for cutting, the brain shows a fairly uniform yellow-brown stain over the entire surface of the cut hemisphere.

For brains not fresh more time and stronger alcohol are necessary.

To Harden Cerebellum.—(Betz.)—The organ should be fresh. Remove the membranes as far as possible. Place in the iodised alcohol, as for cerebrum. By the second or third day the remainder of the membranes can be removed. Add fresh iodine as soon as the liquid changes colour. The preparation remains in this solution 7–14 days more. Preliminary hardening is usually complete by this time. Transfer to solution potass. bichrom. 3–5 per cent., where it remains till fit for cutting.

For pons and medulla the same fluids are used though less time is needed.

The above processes are suitable for obtaining large naked-eye sections. They are not adapted for examination of the cortex, interfering with subsequent staining.

To Harden Large Segments of Brain.—(Hamilton.)—Slice the brain into segments about inch thick longitudinal or transverse, as desired. Leave the membranes *in situ*. Place the segments flat (not one above the other) in a vessel padded with cotton-wool, and containing the following fluid : Müller, 3 parts; methylated spirit, 1 part. In about three weeks transfer to the following solution : ammon. bichrom., 1 gram ; water, 400 ccm. In this they lie one week, after which transfer to 1 per cent. sol. of the same salt; use this for one week. Finally, place in 2 per cent. sol. of the same; here the segments remain until

fit for cutting. A solution of chloral hydrate, 12 grs.– ʒi. is recommended for preserving them in. The method is also very serviceable for ordinary pieces of moderate size.

Vassale (*Rivista Sperimentale*, vol. xvii, fasc. iv) hardens large segments satisfactorily in a mixture of Müller's fluid and conc. aq. sol. zinc chloride (sufficient of the latter added to cause the organ to float). In a few weeks slices 3–4 cm. thick may be cut, passed through celloidin in the usual way (*see* "Celloidin Method"), and microscopical sections made.

A cerebral hemisphere, cerebellum, or large segment of brain may also be hardened very well by either of the two methods next mentioned.

To Harden Pieces of Moderate Size.—[Methods in use at W. R. Asylum; suitable for general purposes.]

(*a.*) Remove a portion of brain, resembling as nearly as possible the size and shape of the sections desired. Place in methylated spirit, or in alcohol 80–90 per cent. for 24–30 hours. (If, however, it is desired to stain the medullary centre of a gyrus, or the intracortical medullated meshwork, the piece should be placed in chrome at the outset; a 1–2 per cent. solution bichromate of potash, frequently changed and gradually increased to 4 per cent.) If any further modification of size or shape be required the piece of brain will be found in a suitable condition for cutting on removal from spirit. Transfer from spirit to potass. bichrom. sol., 4 per cent. Change weekly; 6–7 weeks necessary for hardening. When hard, keep in a weak solution of potass. bichrom. (½ per cent.), to which a little camphor or carbolic acid may be added.

The preliminary use of alcohol serves to prevent

maceration of the deeper parts of the tissue and to hasten hardening.

(*b.*) Place the piece of brain in alcohol (as in [*a*]) for twenty-four hours; transfer to Müller's fluid. Renew this after three days, and in three days more substitute a 2 per cent. sol. pot. bichrom. At the end of the second week, change to 4 per cent. sol. pot. bichrom. In another week replace by a sol. chromic acid, $\frac{1}{4}$ per cent., which is gradually increased in strength. In this hardening is completed. Or the chromic acid may be added to the pot. bichrom. solution both in this and the first method, to hasten hardening. Add 20–30 drops of 1 per cent. sol. chromic acid to 500 ccm. of the bichromate solution.

It is also a good plan to supplement the use of chrome-solutions by after-hardening in alcohol. If carmine stains are to be employed afterwards, the alcohol had better not be used.

Remove the tissue, which is already fairly firm, from the chrome-solution to running water: here the excess of chrome is removed (24 hours). Transfer to alcohol, 50 per cent. for a day, and gradually strengthen the alcohol until 96 per cent. is reached. Keep the vessels used in hardening in a dark room, so that formation of deposits may be avoided. They should not be in alcohol longer than necessary.

The washing process should be omitted if it is proposed to stain the medullary sheaths of nerves afterwards by Weigert's hæmatoxylin-method, which depends upon the formation of a chromium lake in the myelin.

Other Fluids Employed for Hardening Pieces of Moderate Size, with Methods of Use.

Potassium Bichromate and Chromic Acid.—Keep the piece of brain in methylated spirit twenty-four hours, and transfer to following solution: chromic acid 1 part, pot. bichrom. 2 parts, water 1,200 parts. Change the liquid at the end of eighteen hours, and then once a week. If the tissue is not ready for cutting at the end of six weeks, place it in $\frac{1}{6}$ per cent. sol. chromic acid for a fortnight, and then in rectified spirit.

Ammonium Bichromate.—After methylated spirit—twenty-four hours—transfer to 2 per cent. sol. ammon. bichrom. Change at the end of first, third, and seventh days, and thereafter at the end of each week for five weeks, using a 4 per cent. sol. for the last fortnight. Finish in chromic acid solutions, if necessary, or add chromic acid $\frac{1}{4}$ per cent. to the ammon. bichrom. solution.

This is preferred to the ordinary potass. bichrom. solutions by some, on the ground that it hardens more energetically, and preparations treated by it stain better.

Erlitzky's Fluid.—This hardens very rapidly. Many state that the results are as good as with the ordinary (slow-acting) reagents; others believe them to be inferior; and amongst these is the writer. A piece of tissue about 1 cm. by $\frac{1}{2}$ cm., and $\frac{1}{2}$ cm. in thickness, is placed direct in the fluid, composed as follows:—

Pot. bichrom, 2·5 gram.
Cupri. sulphat., 0·5 gram.
Water, 100 ccm.

It is best to make this fresh each time; filter before

use. In an incubator (38–40 degrees C.), hardening is complete in 4–5 days; in the cold, 10–12 days. Change the fluid every two days. Wash the hardened tissues in running water till no more colour is given off, then complete hardening in alcohols of increasing strength: 70 per cent., 80 per cent., 90 per cent.—twenty-four hours in each.

Erlitzky's fluid produces more shrinkage of tissues than the solutions of potass. bichrom. ordinarily used; also deposits in the tissues. The latter may be removed, at any rate partly, by washing with warm water, or water slightly acidified by HCl., or by treating with chromic acid sol. 0·5 per cent. before transferring to alcohol.

Use of Alcohol in Hardening.—Though the chrome-salts have replaced alcohol as general hardening reagents it must be allowed that the structure of the nerve-cells is indifferently shown in specimens hardened in chrome. For the demonstration of the nerve-cell and axis-cylinder alcohol is much superior. Nissl was one of the first to insist upon the superiority of alcohol for these purposes; upon the recognition of this fact is based his method of staining the nerve-cell, described in the sequel. On the other hand, when it is desired to investigate medullated nerve-fibres the use of alcohol is quite contra-indicated, chrome-solutions must be employed. Again, for the fixation of cell-nuclei there are reagents superior to alcohol.

Three classes of hardening or fixing media are therefore required in the investigation of brain-structure as at present carried out: chrome-salts for the nerve-fibres, and for "general survey" purposes; alcohol for the cell-structure; and some fixative-fluid for nuclear structure. [Examples of the last class are given later.]

Alcohol is used in the proportion of about 96 per cent. Small pieces (1–2 ccm.) of fresh brain are placed in this. Change the fluid in about two days; absolute alcohol may then be used. The piece is ready for cutting in 3–4 days. Fine structural details appear to be better preserved by this plan than by that of passing from weak to strong solutions. It is best to cut sections as soon as the tissue is hard enough.

Osmic Acid (Exner's Process: staining combined with hardening).—Excise a portion of quite fresh brain about 1 ccm. in size. Place in ten times its volume of osmic acid sol. 1 per cent. Keep in the dark. Change the fluid after two and four days respectively. Suitable hardening and staining is obtained in 5–10 days. Wash well in water. For cutting sections, fix the tissue to a piece of cork by means of celloidin solution or gum—hardened by means of alcohol; the cork can then be held in the well or clamp of a microtome and sections cut into alcohol. They must be very thin. Place them as soon as possible in glycerine, and thence transfer to slide. As they lie upon the slide add to the glycerine a drop or two of strong ammonia (liq. ammon. caust. 1, aqua 50). Take up superfluous fluid with blotting paper, and as soon as the ammonia has exerted its clearing action adjust the cover-glass. Examine forthwith, as the sections show to greatest advantage now. These preparations are only temporary. With a view to preserving them Exner surrounds the cover-glass with a soluble silicate of glass ("water-glass"). The brain-structure swells up much with ammonia, so that the diameter of the section becomes increased.

By this method the course of the medullated fibres (stained dark-grey) in the cortex can be traced. It has

practically been replaced by Weigert's method. [*See* "Staining Methods."]

Ranvier conducts the process as follows:—Quite small pieces of cerebral cortex are placed in osmic acid sol. 1 per cent. for about twenty-four hours. They should be black throughout. Make thin sections. Place one on a slide and cover with a drop of ammonia. The section swells up and the medullated fibres become distinct. Expose the section to the vapour of osmic acid (placing it over the mouth of a bottle containing the reagent). Remove in half hour or so. Mount in glycerine.

Marchi's Method (Staining combined with hardening).—This very important method depends upon the staining of products of degeneration. Small pieces in which there is reason to believe that degenerate medullary sheaths are present [it is stated that the degeneration must not be more than 2–3 months old; the method is principally used after experimental injury to nerve-tracts], are hardened for eight days or longer in Müller, and then cut into quite small portions (1 ccm.). These are placed for 5–8 days in a mixture of two parts Müller's fluid and one part osmic acid sol., 1 per cent. (Marchi's fluid). After thorough washing they are ready for cutting. Pass through alcohol and imbed in celloidin in the ordinary way; cut, dehydrate, clear, and mount in balsam. A degenerate nerve-tract is indicated by black myelin-clumps and detritus. Normal tissue is light-brown, or a grey colour. Fatty changes in the walls of blood vessels and in ganglion-cells are well shown. The method affords a positive of the picture furnished by Pal's process (*see* "Staining Methods"). It is the best method of tracing degenerate nerve-tracts.

Double staining by carmine or other suitable dye is possible.

Sublimate followed by Alcohol has been recommended for ordinary hardening purposes. Pieces about 1 ccm. in size are placed in a 7 per cent. aq. sol. of sublimate for 5–9 days, and then in alcohols, 50 per cent., 70 per cent., 96 per cent., twenty-four hours in each [Diomidoff]. Deposits of mercury are very liable to occur in the tissues. These may be dissolved out by prolonged washing in water, or in 5–10 minutes by the use of Lugol. [Water 100 parts. Potass. Iod. 6 parts, Iodine 4 parts.—Bolles Lee.]

Or the sublimate-solution given under the Fixation-Methods may be employed, in the manner there described. With this solution deposits seldom appear in the sections. The sublimate method produces more shrinkage than occurs when chrome alone is used, from the outset.

Sections are stained diffusely by the ordinary dyes—anilin blue-black, hæmatoxylin, carmine. The only method of getting a stain satisfactorily limited to the cell-elements is to overstain in one of the anilin dyes (other than anilin blue-black and anilin blue), and subsequently differentiate in alcohol. This procedure involves the risk of partially decolourising the body of the nerve-cell. The writer has obtained good specimens by means of Toluidin-blue [*see* "Anilin Stains"], but is unable to recommend the sublimate method for general work.

IV.—FIXATION-METHODS.

Sometimes it is necessary to examine the finest structures of the cortex (such as the structure of the protoplasm of nerve-cell, and that of the nucleus), especially during developmental changes and in states of inflammation. Under these circumstances some method must be employed by which the parts can be fixed in the state natural to them during life, and also hardened, so as to prevent changes of form subsequently. Fixation-Methods fulfil these conditions. The tissues to be fixed must, of course, be as fresh as possible—taken from a living animal, or within one-half hour of death. Obviously, therefore, these methods can only under exceptional circumstances be applied in the case of man. For the study of the nucleus (nuclear figures, nuclei in process of division) rabbits, rats, cats, &c., at different periods of life may be employed; and the cortex should be examined after experimental irritation.

The following practical points should be considered in employing fixatives.

Tissues should be either from the living body or as fresh as possible. As the fluid has to penetrate the tissue rapidly, the latter should be thin (say 5 mm.). In superficies the object should be 1–2 cm. about. If

large objects have to be worked with, incisions should be made, to allow penetration.

The quantity of fixing agent employed should be many times the volume of the object.

The fluid should be changed if it becomes turbid.

Penetration is facilitated by heat. The incubator may be employed.

After fixation, wash the tissue thoroughly in order to remove, as far as possible, traces of the fixing agent. In most cases water may be used for the purpose.

After washing out, the tissue is commonly after-hardened in alcohols of increasing strength.

The following are some of the fixing fluids which may be employed, with their methods of use. [The times given refer to fixing in the cold.]

Flemming's Chromo-Aceto-Osmic Mixture.—The subjoined modification by Friedmann gives good results. It contains less osmic acid than the original mixture, and so is less liable to darken the ground substance.

Osmium, 1 per cent. sol.	½ part.
Chromic Acid, 1 per cent. sol. . .	7 parts.
Glacial Acetic Acid	0·3 part.

Pieces of tissue remain in this 10–24 hours; wash for a short time in water, and harden subsequently in spirit 2–4 days. Sections stain rather better when the tissue has been some time in spirit.

Fol's Modification of Flemming.

Osmic Acid, 1 per cent.	2 parts.
Chromic Acid, 1 per cent.	25 „
Glacial Acetic Acid, 2 per cent. . .	8 „
Water	68 „

Pieces remain in this twenty-four hours or so, are then carefully washed in water, and transferred to alcohol 80 per cent., where they remain till used.

Rabl's Fluid.

Chromic Acid sol. 0·3 per cent. .	200 ccm.
Conc. Formic Acid	4–5 drops.

Pieces remain in this 12–24 hours. Wash well in water. Successive hardening in alcohols 30 per cent., 60 per cent., 96 per cent.

Merkel's Fluid.

Chromic Acid sol.	1:400	aa
Platinic Chloride sol. . . .	1:400	

Pieces remain in this 4–6 days. Wash out with alcohols of increasing strength, 50 per cent. to 70 per cent.

Benda's Fluids.

(*a*) Nitric Acid	10	parts.
Water	90	„
(*b*) Pot. Bichrom.	1	„
Water	3	„

(*b*) is gradually increased in strength until the proportion is pot. bichrom. 1 part, water 1 part. After remaining 24 hours in (*a*) transfer directly (without washing) to (*b*), in which pieces remain 10–14 days. Wash well in water. Alcohols of increasing strength to 96 per cent.

This method is not adapted for embryonic structures, but otherwise is very satisfactory.

Alcohol (96 per cent.).

Pieces remain in this 1–3 days. Keep, if not cut at once (as they had better be), in alcohol 90 per cent.

Nitric Acid (10 per cent. aq. sol.).

Pieces remain in this 1–3 hours. Wash. Alcohols, 70 per cent., 80 per cent., 90 per cent.—24 hours in each.

Sublimate Solution.

Sublimate . . . . . 7·5 grm.
Salt solution, 0·5 per cent. . . . . 100 ccm.
Dissolve by heat.

Pieces remain in this about 24 hours. Wash very thoroughly in water. Pass through alcohols, 30 per cent., 70 per cent., 96 per cent.—24 hours in each. Unless thoroughly washed deposits of mercury occur in the tissue.

Vignal, in his work upon the development of the elements of the nervous system, found that in the earlier phases of development Flemming's mixture, and other of the more commonly employed fixatives, gave good results. But from the fifth to eighth month, and in still later stages, all the reagents ordinarily employed for the fixation of adult tissues proved useless. The only method found to be satisfactory was the following: Pieces of brain, 2–5 mm. in thickness, are placed in one-third alcohol (Ranvier's), being detached from the knife by simple agitation in the fluid [no handling]. They remain in this twenty-four hours. They are then cut into fragments about 3 mm. in thickness, and these are placed in a solution of picrocarminate of ammonia, 1 per cent., for 2–3 days. This is then removed by pipette, and replaced by osmic acid, 1 per cent. sol., which is left in contact with the tissue for twenty-four hours. Wash in water, then in alcohol 90 per cent.; absolute alcohol. Cut in celloidin. Sections should be very thin. Mount in dammar.

Tissues, fixed after the methods mentioned, may be cut in wax or celloidin, according to the processes described in the sequel, and the sections stained with safranin, gentian-violet, or hæmatoxylin. Other anilins than those given may be employed, but the latter are most commonly used. The hæmatoxylin may be Ehrlich's, or some one of the solutions given in the section on Staining; and the method of use is as there described. Safranin or gentian-violet is used as follows: [A safranin suitable for staining nuclei should be obtained of Grübler, or Münder. For addresses, *see* section on "Staining."] Make a concentrated solution in absolute alcohol and dilute with one-half water [Flemming] or a mixture of equal parts conc. alc. sol., and conc. aq. sol. (Babes). Stain 12–24 hours. Wash lightly in water, and transfer to absolute alcohol—strong spirit (95 per cent.) will do. The colour escapes in clouds. When their formation is about to cease (in case of safranin, the section becomes of a purple hue), washing-out is stopped at once by transferring the section to xylol. Here it is cleared. Mount in xylol balsam. If the colour is insufficiently abstracted by alcohol, acid-alcohol is momentarily used. (Conc. HCl. 1 ccm., alcohol 70 per cent. 100 ccm. Two or three drops of this to a watch-glassful of alcohol.) Transfer the section finally to absol. alc. before using xylol. This should also be done when spirit has been used for decolourisation.

Sections of tissues fixed in sublimate are usually stained by the Biondi-Ehrlich method. The stain requires careful preparation, and it is best to obtain it from one of the agents mentioned above. Stain 6–24 hours in the slightly diluted solution. Wash

rapidly in alcohol, 90 per cent.; then successively in abs. alc., xylol, xylol-balsam. Toluidin-blue also gives good results. (*See* "Anilin Stains.")

For tissues fixed in alcohol, Rehm recommends the following method of staining, as suitable for showing nuclear structure. This is more difficult with tissues fixed in alcohol than with those fixed by the other fluids given above. Sections are brought out of alcohol into a carmine-solution. Rehm has usually employed a 1 per cent. sol., with liq. ammon. caust. 1 ccm. Here they remain about five minutes, and are then transferred to acid alcohol (nitric acid 1 ccm., alcohol 70 per cent. 100 ccm.), where they remain 3–4 minutes. Wash in water, then in alcohol. Transfer to cold aq. sol. methylene blue, 0·1 per cent., where sections lie not more than half minute. Remove excess of stain in alcohol. Origanum oil, mount in colophonium (benzole or xylol solution). Sections appear a blue-violet. Ground-substance is pale-rose, ganglion-cell bodies are blue, nerve-cell nuclei red, the fibrils of the nuclear network appearing distinctly—of this colour. Nuclei of connective-tissue and blood-vessels are stained shades of blue and violet.

By the above method the structure of cell-nuclei in particular may be examined. In human brain, affected by various degrees of inflammation, and about the inflammatory foci produced in the brains of animals by irritants, changes in the nuclei of cells have been described by various writers: karyokinesis, multiplication of nuclei, increase of their chromatin. The conditon first mentioned is especially difficult of demonstration; one often fails to observe it in cerebral lesions theoretically well-adapted for the purpose, even

when strict precautions have been taken in the preliminary treatment of specimens.

Mention may here be made of the method of examining cerebral tissue after *digestion,* by means of acidified solution of pepsin or alkaline solution of trypsin. Portions of cerebrum and cerebellum, fresh, or hardened by the usual means, are kept in the digestive fluid in an incubator at 38 degrees C., for some days if fresh, 3–4 weeks if hard. Move them about in the fluid, and change occasionally. Subsequently the pieces are hardened (if fresh) and dehydrated, cut as usual, and sections stained by the various dyes.

No important results have yet accrued from this method of examination, as far as the writer is aware.

Recognising that the myelin obstructs the diffusion of stains in nerve-tissue, certain investigators have endeavoured to "*demyelinise*"—to dissolve the myelin —as far as possible, before staining the axis-cylinder and cells, in those cases in which a nuclear and connective-tissue (as opposed to a myelin) stain is required. The method appears to have been applied mainly to spinal cord. Paladino hardens small pieces of tissue in chrome-salts, as usual, and then boils them successively in a mixture of absolute alcohol and benzole, in benzole, and in alcohol 96 per cent. The pieces remain about one hour in each of these media. They are then stained, dehydrated, and cut in celloidin. For staining, Paladino uses his iodide of palladium method. [*See* "Staining Methods," where the process is again referred to.] By this means, important observations have been made upon the neuroglia—the framework of medullated fibres, and the structure of the axis-cylinder. [Paladino: *Archives italiennes de Biologie*, t. xix, fasc. i, 1893.]

The tissues being properly hardened, the examination may either be at once proceeded with, or, if that is inconvenient, and they have been hardened in chrome-salts, tissues may be kept till required in potass. bichrom. sol. 0·5 per cent. It is especially desirable that they should remain until used in weak chrome solution if sections are to be stained by Weigert's hæmatoxylin process. Alcohol-hardened tissues, if not cut as soon as ready—as is desirable—may be kept in alcohol 90 per cent. Occasionally a preservative-fluid, such as chloral (12 grs.–℥i water) is used for keeping hardened segments in. If sections are to be obtained by the freezing method the hardened tissues may be kept (after washing in water to remove spirit or excess of chrome) in the gum and syrup mixture mentioned below, for an indefinite time. If by the celloidin method, they may be kept in the weak chrome solution as usual, or in a thin solution of celloidin in alcohol and ether.

V.—IMBEDDING, INFILTRATION, AND SECTION-CUTTING.*

To Obtain Sections from Large Pieces of Brain, a Cerebral Hemisphere, or the Whole Brain.—Simple imbedding may be employed, as described in the sequel, for small objects. A specially constructed well-microtome and knife will be needed [*see* Bevan Lewis, *Brain*, Jan., 1882]. Cutting is done either under alcohol or water, the microtome resting in a zinc or copper basin holding the fluid. Remove sections on sheets of paper, on which they may remain during the subsequent steps of preparation.

The organ may also be cut after the freezing methods about to be described. A special microtome has been devised by Hamilton [*see Text Book of Pathology*, vol. i, p. 65], for cutting sections of entire organs, such as the brain, in a frozen state. It is made by Gardner, Edinburgh. The freezing mixture consists of snow and salt. Soak the organ before freezing in his freezing fluid "B" (*v. infra*).

Bruce has also introduced a microtome for cutting sections of the entire brain after freezing, made by Frazer, 7, Lothian Street, Edinburgh. [*See Journ. Roy. Microscop. Soc.*, 1888, pt. 5, p. 837.] Gudden's microtome is also adapted for cutting the whole brain. (A well-instrument for imbedding in wax, &c. Cutting

* Previously hardened tissues are alone referred to.

is done under water. Supplied by Katsch, Munich.) See also the method of Deecke (*op. cit.*, 1888, p. 1051), and Byron Bramwell, "A Method of preparing Large Naked-eye Sections of Brain." *Brain*, Jan., 1888.

By means of the celloidin-process sections may be cut from large sized pieces of brain.

To Obtain Sections from Pieces of Moderate Size.

The Freezing Method.—This may be described as the simplest and most generally suitable. Remove the hardening fluid from the tissue, especially if spirit has been used, by immersion in running water for 12–24 hours. When sections are to be stained by Weigert's hæmatoxylin method the washing-out process should be of short duration. Transfer to a mixture of mucilage (B.P.) 5 parts and syrup (B.P.) 4 parts. If this is found to freeze with too much difficulty, diminish the proportion of syrup. In this mixture tissues remain 24–48 hours or longer. As stated, they may be kept in it indefinitely; add a few drops of carbolic acid for each ounce of the fluid. Freezing may be brought about by the ice and salt mixture or ether-spray. Amongst the available microtomes are Cathcart's (ether), Bevan Lewis's (ether), Rutherford's (ether or ice-and-salt), and Williams's (ice-and-salt, with special knife). The knife described in the Freezing Method may be used, or, with Cathcart's instrument, a plane iron. Remove the gum and syrup from the exterior of the piece to be frozen and fix the latter to the freezing-plate by gum-mucilage; also paint round with the same. If Rutherford's instrument is employed gum is poured into the well, and the tissue immersed in it and held with forceps until fixed

by the freezing action of the surrounding ice. Smear a little gum and syrup upon the part of the knife on which sections accumulate. The mass should cut like cheese. Sections are received into tepid water, where they remain until the gum and syrup mixture is washed out. (2–4 hours.) If not to be examined forthwith, sections may be preserved in a mixture of glycerine and water, equal parts, to which is added a drop or two of carbolic, 1–20; or in weak spirit (methyl. spirit, 1 part; water, 2 parts), after thoroughly washing out the gum.

To ensure against any risk of sections splitting up in water the tissue may be imbedded in celloidin before freezing in gum. The following plan [Hamilton] may be employed. The piece of hardened brain is placed in methylated spirit for 3–4 days; change daily. Transfer to a mixture of abs. alcohol and ether equal parts; here the tissue lies two days. Make a solution (syrupy consistence) of celloidin in equal parts of alcohol and ether. In this the tissue remains at least four days. Place the piece now in a paper boat into which the celloidin is poured; it hardens. Hardening can be hastened, when it has already reached an appreciable degree, by methylated spirit. When complete, the piece of brain surrounded by the hard celloidin is soaked in water twenty-four hours, and kept in freezing-fluid "A" or "B" (*v. infra*) for several days. The fluid penetrates the tissue more quickly if a warm chamber be employed. The piece is now ready for freezing and cutting.

Hamilton's Freezing Fluids.—D. J. Hamilton recommends the following fluids, by the judicious employment of which the integrity of the tissues is well preserved. In the first place make a *syrup*,

28½ grm. pure sugar to 30 ccm. water. Saturate whilst boiling with boracic acid. Filter cold through muslin. Next make a *mucilage* of gum acacia in cold water, 45·6 grm. of gum to 2,400 ccm. water. Saturate whilst boiling with boracic acid; filter as before.

Fluid A.—Syrup, 4 parts; mucilage, 5 parts; water, 9 parts. Boil; saturate whilst hot with boracic acid. Filter cold through muslin.

Fluid B.—Take of A 2 parts; and syrup, 1 part.

Fluid C.—Syrup, 4 parts; mucilage, 5 parts.

These three fluids freeze with different degrees of hardness. "A" is the easiest (freezes hardest), "C" the most difficult to freeze. "A" is sufficient for most purposes, but certain delicate tissues likely to be injured by ice may require "B," or even "C." With these there is no risk of injury during freezing. Pieces should be allowed to soak in the fluid to be used for freezing for a period dependent on their size; in any case, not less than one week. But hardened tissues may be kept permanently in these mixtures, after the hardening fluid has been washed away by water.

Section-cutting by the Sliding Microtome*—The Celloidin Method.—If the tissue to be cut is of good consistence after hardening is complete it is placed in running water, supposing chrome has been employed, until the excess of the hardening reagent is washed

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* This instrument can be obtained from the following makers:—Jung (Heidelberg) supplies the Thoma microtome; Becker (Göttingen); Katsch (Munich); Reichert (Vienna); Schanze (Leipzig). All are obtainable from G. Koenig, Berlin, N.W. Dorotheen Strasse, 29. See his catalogue for comparison. These microtomes can be procured adapted for ether-freezing as well.

out; then dehydrated in alcohol. It is now mounted on a cube of wood of suitable size and the latter is fixed in the clamp of the sliding microtome. Fixation is brought about through the medium of gum; pour a little on to one surface of the cube, apply the tissue to it, and place the whole in methylated spirit for twenty-four hours. A thick solution of celloidin serves equally well; to harden this use alcohol of about 80 per cent.—after allowing the solvent to evaporate for a quarter-of-an-hour or so. Or use an adhesive fluid made as follows: Allow some gelatine to swell in water for some hours, pour off the water, and mix together by heat equal vols. of this gelatine and glycerine. Add a little camphor to prevent decomposition, filter through linen, and allow filtrate to set. The substance may be kept in stock. At the time of use, take a small piece of the jelly on the point of an old knife and liquefy over a flame. Smear the liquid over one surface of the cube of wood and adjust the preparation. Place the whole in alcohol, where the jelly quickly sets very hard [*v. Kahlden*].

If for any reason a good consistence for cutting has not been obtained* (perhaps, for example, certain parts have been softened by disease or become friable by long keeping) the tissue—after washing out and dehydration as before mentioned—should be infiltrated by and imbedded in *celloidin*.† [Prepared by Schering, Berlin, and obtainable from manufacturing chemists.]

The Celloidin Method.—Make three solutions of celloidin in a mixture of absolute alcohol and ether, equal parts. The first should be thin, the second of

* Or if it is of some size.

† Collodion is equally efficacious and cheaper.

the consistence of thick syrup, and the third rather thicker than the second. The piece to be treated, after thorough dehydration in absolute alcohol, is placed in a mixture of absolute alcohol and ether, equal parts. It lies at least two hours in this. The mixture is more particularly to be employed with objects but slightly permeable; for others it is not essential. Transfer from this mixture to the thin solution of celloidin. Here the tissue remains twenty-four hours, or as much longer as one pleases. Transfer to the second, thicker solution, for twenty-four hours. If there is no hurry pieces may remain several days in each solution. In considering the time needed the size of the piece has, of course, to be allowed for. For cutting, fix the tissue on a cube of wood of suitable size with the thickest solution. Expose to the air for an hour or so, to allow of evaporation, and then place the whole in alcohol, 80 per cent. Here the celloidin is rendered sufficiently hard in one or two days. The preparation, so fixed, may be kept in alcohol of 70–80 per cent. until needed. Instead of alcohol, pure chloroform may be used for hardening the celloidin. It is sufficient to expose to the air for a few minutes only before immersing in the chloroform, which sets the celloidin in a few hours. By using this in place of alcohol a greater transparency of the celloidin appears to be secured.

To cut sections, fix the cube of wood in the clamp of the microtome and adjust the knife to form an acute angle with the preparation; the best position for the particular case will be found by practice. Keep the upper surface of the knife thoroughly moist with spirit (about 80 per cent.) during cutting, also that of the preparation. For removing the section from the knife

a piece of thin paper (toilet) is convenient. Float the sections into spirit (80 per cent.)* Here they remain until they are to be stained. Some prefer to work with the whole microtome under spirit. The best plan is probably to have a tray holding spirit arranged alongside the block with its specimen, so that the latter is immersed in spirit when cut.

Under quite exceptional circumstances the sections may be so brittle that it is advisable to "collodionise" them. Pass a camel-hair brush which has been dipped in a very thin solution of celloidin (keep the solution very thin) over the surface of the preparation; allow the collodion to dry (a few seconds suffice); cut the section.

In certain cases, especially when anilin dyes are to be used, it is desirable that the celloidin should be removed from the section (if the tissue is not too friable) before staining. The section is transferred to absolute alcohol for a short time, thence to a mixture of abs. alc. and ether, in which it remains 10–15 minutes, or to clove-oil (momentarily), then back again to abs. alcohol, and thence to water. After this it is stained.

To Mount a Series of Celloidin-Sections.—This is occasionally necessary. Weigert's process is very satisfactory. Proceed as follows:—Cut strips of toilet-paper of about twice the width of the sections. Cut a section, taking care that there is not sufficient alcohol on the knife to cause the section to float. Soak one of the strips in the alcohol in use and lower it, stretched, on to the section lying on the knife. The section

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* Absolute alcohol dissolves celloidin.

adheres to the strip and is removed with it. The first one taken should be at the left end of the strip; the remaining sections are taken in order, from left to right. After each has been taken up, and also when the paper-lifter has its proper complement, and whilst other strips are in preparation, lay the strip, sections uppermost, on a moist surface prepared by saturating a layer of closet-paper—placed on several layers of blotting paper—with alcohol. Take a number of glass plates and spread out on each a thin layer of collodion; allow it to dry, lay the paper slips, sections downward, on the glass plates; press gently, the sections adhere to the collodion. Remove the paper. Draw off excess of alcohol with blotting paper, but do not allow the sections to become quite dry. Now pour collodion over them, and spread it out in a thin layer.

The plate can now either be put away in 90 per cent. alcohol [this is better than the 80 per cent. usually recommended; there is less liability to cloudiness afterwards] or brought into a staining fluid—for example, Weigert's hæmatoxylin (*see* "Staining Methods"). In this the collodion becomes quickly detached from the glass—especially if the incubator is employed—holding the sections fast. Supposing Weigert's hæmatoxylin process to be followed, the sections are stained, differentiated, and washed, as usual. Whilst in water cut the series into the desired lengths for mounting. Then dehydrate in alcohol 90–95 per cent., clear in a mixture of xylol 3 parts, pure carbolic acid 1 part. Mount in xylol-balsam.

It may here be mentioned that clove-oil dissolves celloidin and therefore should not be used for clearing celloidin sections. Besides the xylol-carbolic mixture

any of the following clearing agents may be used for such sections: origanum-oil, cedar-wood oil, bergamot-oil, chloroform, benzine, xylol.

Another method of mounting series of celloidin sections is the following [Minot's Shellac Method]:— Sections are stained, and dehydrated thoroughly in alcohol. Place them on the slide in the desired order, keeping them covered with alcohol. Drain off the alcohol slowly. Cover the sections with 10–12 per cent. sol. refined shellac, and expose the slide at once to 30–40 degrees C., until the shellac is dry. Clear in clove-oil. Balsam.

In place of celloidin, *photoxylin* is now largely used. It possesses all the merits of celloidin and has in addition the advantage of greater translucency. In the investigation of small and lightly-stained objects, this is a point of importance. Photoxylin is stated to be allied chemically to celloidin. It has the appearance of fine, pure cotton-wool, is soluble in equal parts of alcohol and ether, and is employed in precisely the same manner as celloidin. [Obtain from Grübler or Münder].

The Paraffin Method of Infiltration. — Objects imbedded in and infiltrated by paraffin can be cut by the sliding microtomes. In this case the knife is fixed at right angles to the main body of the instrument, and its movements are quick and of small amplitude. A ribbon of fair length can thus be obtained. Special instruments for cutting in paraffin are made by the Cambridge Scientific Instrument Company (Cambridge), and by Zimmermann (Leipzig—Minot's instrument). The latter can be obtained through R. Kanthack, Golden Square, London.

The paraffin method is as follows: The object should be small. Pieces, 1 cm. by 5 mm.–1 cm. may be taken. After hardening and, if chrome-solutions have been used, washing out excess of chrome, dehydrate thoroughly in abs. alc. (8–24 hours). Then place in some medium which mixes alike with alcohol and paraffin—for example, xylol, toluol, benzol, chloroform, ess. turpentine, or cedar-wood oil (the last a very good medium). Here the piece remains 4–6 hours. Transfer to a mixture of cedar-wood oil (or other solvent above mentioned) and paraffin about equal parts. Keep in thermostat at a temperature sufficient to maintain fluidity of the mixture (4–6 hours). Transfer to pure paraffin kept in a paraffin oven at a temperature as little above the melting point of the paraffin as possible. One or two changes of paraffin are advisable, in order to get rid of the solvent. The melting point of the paraffin is a very important matter, from the point of view of section-cutting. Obtain two sorts of paraffin, of different melting-points—high and low—and mix to secure a mass of suitable consistence for cutting in summer or winter, as the case may be. It should, of course, be harder for the former than for the latter. The paraffin of low melting point (soft) will melt between 45 and 48 degrees C., that of high melting point (hard) between 55 and 60 degrees C. For summer a paraffin melting at 52–54 degrees C., for winter a mixture melting at 48–50 degrees C. may be used. [A melting point suitable to the temperature of the laboratory will be found by experience]. The object remains in the paraffin-bath 4–5 hours. It may be said here that the length of stay in the various media depends on the size and permeability of the

piece of tissue. When thoroughly infiltrated, fill a pill-box of suitable size with the paraffin, having previously smeared some glycerine round the sides and bottom of the box to facilitate removal of the mass. Fix the object at the bottom of the box in suitable position, and then dip the latter, whilst the paraffin is still quite fluid, in cold water nearly up to its brim. It is important that the paraffin should set rapidly. When the mass has thoroughly set, cut away the surrounding card-board, and, with a scalpel slightly warmed, shape out a cube containing the object. Cut close up to the latter, leaving only a narrow casing of paraffin. The part of the object next to the bottom of the pill box should be cut first; fix the end of the block remotest from this to the holder of the microtome, which has previously been smeared over with a layer of paraffin. Fixing is performed by passing a warmed metal section-lifter between the two paraffin surfaces on its withdrawal adhesion is easily affected by slight pressure. The block should be quite firm before cutting is attempted. Fill up any small cavities which its sides may present by means of a warm needle. Cut ribbons of sections with the knife dry. The cutting of ribbons is facilitated by smearing some paraffin of low melting-point over the side nearest in cutting and that opposite to it. If the paraffin is too hard a good way of softening it slightly is to throw upon the block the rays from a parabolic reflector, placed at a suitable distance. "Rolling" of sections can, with care and patience, often be prevented by flattening each section against the knife, in process of cutting, with a spatula, or by pressing gently with a camel-hair brush on the upper surface of the block whilst the knife cuts through.

It is frequently overcome by throwing the section as soon as cut on the surface of warm water. Shortly after removal from the water, sections may be dried between two layers of blotting paper. They may be kept as they are in a box until required for further treatment. For subsequent treatment (*see* "Staining Methods").

The rule is to use celloidin for large, and paraffin for small objects. For holding together parts loosened by disease, and for fixation of morbid products—as exudates—the celloidin method should be used. The objection to the paraffin process for tissues of this kind is that the imbedding paraffin has to be removed before the sections can be prepared for examination. The only available procedure, supposing paraffin to be used for such tissues, would be to fix the sections on a slide by one of the methods in vogue, remove the paraffin, and stain on the slide. In the writer's experience the results are capricious and unsatisfactory when diseased sections are stained in this way.

Apart from this, the celloidin process is better adapted for delicate structures, in which it is desired to examine fine details and morbid changes than the somewhat severe paraffin-infiltration process.

The preliminary staining of pieces *en masse*—one of the main advantages of the paraffin method—the writer is quite unable to recommend in the case of diseased cerebral tissue.

When, however, the object is merely to demonstrate the general structure of the healthy cortex—as for class purposes—the paraffin method may be employed, and the tissues stained in mass, or as sections fixed to the slide. But the practice of staining even healthy tissues

(whatever their nature) in mass, is now very generally regarded as unsatisfactory.

Simple Imbedding Method.—In this the following media may be employed:—

1. White wax and olive-oil, equal parts.
2. White wax and cacao-butter. Vary proportions as desirable.
3. Paraffin, 5 parts; hog's lard, 1 part.

That first mentioned is most commonly used. Equal parts of wax and oil produce a mass of good consistence for cutting at any temperature between 54 degrees Fahr. and 66 degrees Fahr. For temperatures above and below these the consistence is increased or diminished by adding more wax or more oil respectively. In practice, it is found that a good consistence for cutting is obtained by keeping the mass warm in the intervals of use; the tin-pot holding it may be kept on a hot water pipe, or against a stove. Although the heat is only irregularly applied in these ways, a certain elasticity is conferred upon the wax mass, which proves beneficial when the latter is cut.

In this method, Stirling's well-microtome, or other similar instrument, is employed. The knife should have the characteristics described under the Freezing Method (fresh tissues), but it is well to have also a second instrument, of greater length and breadth, for larger sized pieces of tissue. [*See* Appendix, "Laboratory Equipment."]

The object to be cut, which must be of suitable size for the well of the microtome, is first washed free from excess of the hardening reagent, supposing chrome to have been used, and then dehydrated in alcohol. A

few minutes to one-half hour suffice for the purpose. If the imbedding mass is not already fluid from having been warmed as suggested, it is now melted. The screw of the well-microtome is then lowered sufficiently to allow of proper imbedding. The melted mixture is now poured into the well until it is full. At this stage a small block of the solid mass is thrust down to the bottom of the well, and left near its anterior boundary. On it the piece to be cut is to be placed; it must, therefore, be of such height that, when the piece is *in situ*, there is still a depth of 4–5 mm. of the imbedding medium over the latter. These preparations made, remove the tissue from the alcohol, rapidly dry its surface, and fix it upon the block mentioned. The upper surface of the tissue should be well beneath the level of the melted wax. The melted mass is now allowed to set. When set, make a mark over the seat of the imbedded tissue.

The mass contracts in setting. If notably, more oil must be used on the next occasion. But it may be usually fixed firmly in the well by proceeding as follows: Force the mass out, and replace it with a piece of blotting paper applied to the far side. Pour some spirit over the whole; the paper swells, and the plug is firmly fixed.

The wax may now be removed by sweeps of the knife-blade down to the level of the tissue, raising the plug meantime by means of the screw beneath the well. All wax is then picked carefully away with a scalpel from the front and sides of the tissue for a considerable depth, leaving it supported posteriorly only. Whilst cutting sections, the tissue is kept moistened with spirit, and the upper surface of the knife flooded with

the same. The spirit may be contained in a bottle provided with a tap, arranged conveniently, so that, by turning the tap merely, the preparation and knife may be covered with spirit.

Keep the section-plate free from wax and scratches. Sections are cut by a steady, continuous sweep of the knife, from heel to point, away from the operator. They are placed in spirit, where they remain till required for staining. [Not long, if Weigert's hæmatoxylin method is to be employed.]

It will be seen that both by this method, as described, and that in which the piece to be cut is simply fixed in the clamp of the sliding microtome, without preliminary infiltration, good sections can only be obtained from properly hardened tissues.

If need be, however, pieces to be cut in wax may be infiltrated by celloidin (in the manner already described) beforehand.

Pieces may also be fixed to a cork, of suitable size, by celloidin, or other means, and the cork pushed into the well of the microtome. Wax, in this case, is dispensed with.

When the necessary skill has been acquired, cutting in wax may sometimes be of service, where only a few sections at a time are needed. A well-microtome may occasionally be preferred to the instruments in ordinary use at the present day, on the ground of economy. It is quite unnecessary to point out the great superiority, for all purposes, of the sliding microtome over the well instrument.

Treatment of Sections.—The sections obtained by the various methods described will be in water (possibly glycerine and water), or spirit, or enclosed in a film of

paraffin. With the exception of the class last mentioned, they may be simply washed in distilled water before immersion in the stain. As regards the paraffin sections: If the tissue has been stained *en masse* (*v. infra*), it is merely necessary to fix the sections to the slide by one of the methods given below; melt the paraffin by placing the slide for 5–10 minutes in the paraffin-oven (52 degrees C., or so), and then dissolve and remove it by means of warm xylol (or toluol, turpentine, naphtha). The sections, freed from paraffin, and cleared at the same time by the solvent, are now ready for mounting in balsam.

If the sections are to be stained they may either be first fixed to the slide or treated separately. The paraffin is removed by xylol, xylol by absolute alcohol, and this again by water. Sections are now ready for staining—the ordinary dyes (carmine, hæmatoxylin, anilins), may be used. Free sections may also be stained by Weigert's method. After removal of paraffin they are placed in the acetate of copper solution for a few hours. Rinse in spirit (alcohol 70 per cent.), and place in the hæmatoxylin. With the Weigert-Pal method staining tends to be faint. The writer has been unable to stain even healthy sections satisfactorily by Weigert or Weigert-Pal after fixation to the slide.

For staining *en masse* a piece about 1 cm. square and 3 mm. thick should be taken. The following are some of the stains available:—Anilin blue-black—a concentrated sol. in equal parts of alcohol and water. Stains in 2–3 days. Borax-carmine (alcoholic sol., Grenacher's), followed by acid-alcohol for one hour or so. Stains in 6–7 days. Logwood (Ehrlich's); 2–3 days. Alum-cochineal (Czokor); 14–18 days. After staining, the

tissue is dehydrated and passed through xylol (or other solvent) into paraffin, infiltrated by the latter, and sections cut and treated as described above.

[For staining *en masse* with Weigert's hæmatoxylin, *see* description of Weigert's hæmatoxylin process.]

For fixing sections to the slide various media are employed, of which the following may be given.

Meyer's Albumen.—White of egg 50 ccm., glycerine 50 ccm., salicylate of soda 1 grm. Shake well together and filter into a clean bottle.

Clove-Collodion.—(Schïllibaum's Collodion).—One part of collodion is shaken up with three or four volumes of clove-oil.

Either of these should be spread in a *thin* layer on a slide and the sections fixed by light pressure. Place the slide for 5–10 minutes in the paraffin oven, remove the melted paraffin by warm xylol, and proceed as described above.

Altmann-Gaule Method.—In this the fixation depends upon capillary attraction. The slide must be carefully cleaned. Cover it uniformly with distilled water and place the paraffin sections upon the layer of water. Remove excess of fluid by filter paper and place the slide in the paraffin-oven at 50 degrees C. for 24 hours. After submitting the preparations for a few minutes to a rather higher temperature, over the melting point of the paraffin, they may be treated as usual (removal of paraffin, &c.). This method requires some experience and is likely to result in failure at first.

VI.—STAINING METHODS.

The various stains* employed for demonstrating microscopical structure in hardened brain may be conveniently classed as follows :—

Ammonia-Carmine and Carmine Combinations stain nerve-cells (the protoplasm of cell bodies) and their nuclei, axis-cylinders, and connective-tissue structures generally. Ammonia-carmine is rather diffuse, staining the ground-substance, but the other forms are more precise, and might, in fact, be placed under the next heading.

Nuclear Stains (especially *Hæmatoxylin*) pick out the various nuclei present in brain-tissue, staining the protoplasm of cell bodies lightly.

Stains Bringing Out Medullated Nerve-Fibres.

Anilin Stains (single and combined): Some stain nerve-cells and their processes well, but most are nuclear dyes.

Other Combination Stains serve to produce contrasts by staining different tissue elements different colours.

Metallic Stains.

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* All stains should be obtained from a reliable source. The preparations of Grübler and Münder are well known as reliable. The price list of one of these agents should be kept. Addresses: Dr. G. Grübler, Leipzig, Baiersche Strasse, 12. Agent—R. Kanthack, Golden Square, London. Dr. G. Münder, Mikroskopisch-chemisches Institut, Göttingen.

Special Stains (miscellaneous): used according to special methods.

The staining process may be accelerated by warmth; the incubator (T=38 degrees C.) may be used for the purpose. Slow staining, however, is generally preferable; and therefore, unless for some reason the dye penetrates with difficulty, or there is necessity for haste, it is better to stain at the ordinary temperature of the room. The times given with the methods about to be described apply to staining at the ordinary temperature.

To prevent the growth of moulds add a little thymol to the stock solution of stain, unless it contains a considerable amount of absolute alcohol, when this is unnecessary.

Filter all stains before use.

As a general rule it is best to use the stain in a watch-glass, covered to prevent evaporation.

The results obtained with the carmine, hæmatoxylin, and cochineal stains refer to sections from tissues hardened in bichromate of potash, washed free from excess of chrome by water, and after-hardened in alcohol a day or two. [In the case of carmine, control-sections from tissues not subjected to after-treatment with spirit.]

Carmine Stains.

The best carmine should be employed.

Ammonia-Carmine (Beale).

Carmine	10 grains.
Liq. ammon. fort. (B.P.)	½ drm.
Glycerine (Price's)	2 ozs.
Alcohol	½ oz.
Dist. water	2 ozs.

The carmine in small pieces is dissolved in the ammonia by heat; boil for a few minutes. Leave the solution exposed at least one hour, or until the excess of ammonia has evaporated and the odour is faint. Add the glycerine, water, and alcohol; filter. If in course of time the carmine begins to precipitate, add a drop or two of liq. ammoniæ.

For use, dilute this solution with about seven times its bulk of water, and filter. Twelve hours or longer required for staining. Much depends on the length of stay in chrome; the older the preparations the longer staining required. It is a matter of trial. When stained, remove excess of carmine by agitating in water, and place the sections in dilute glacial acetic acid (0·5 per cent.), or in following:—HCl (1 per cent. sol.) 1 part, Sp. vini rect. 2 parts. They remain in the dilute acid about 15 minutes, to remove diffuse staining. Wash in water, dehydrate in absolute alcohol, clear in clove-oil (or creosote or origanum-oil);* mount in canada balsam (benzole or chloroform solution).

Simple Aqueous Ammonia-Carmine (Betz).—This is a solution of carmine in weak ammonia. Carmine is rubbed up with a little water until a thick syrupy mass is obtained; add ammonia, which brings about solution of the carmine, and stir well. Dilute with a large amount of water; filter. The filtered solution is exposed to daylight (sun, preferably), in an uncorked vessel of green glass, until a dirty red precipitate appears. It is now filtered. Allow the filtrate to

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* These are common clearing agents. Others may be used. *See* "Clearing Agents."

stand again, under similar conditions, and when the precipitate again forms it is filtered off. Expose a third time, and filter again if necessary. The fluid is now ready. Keep in a corked vessel. During exposure, a flocculent membrane may form on the surface of the fluid; this probably indicates the presence of a micro-organism. The fluid is, however, none the worse. In fact, the chemical changes which take place in the solution, and with which its merits as a stain are connected, appear to be in part due to the growth of an organism.

Stain for $\frac{1}{2}$–1 hour, more if necessary. Proceed subsequently as in last instance.

Hoyer's Dried Ammonia-Carmine is a useful preparation. Make an aq. sol. of ammonia-carmine as follows:—Carmine 1 grm., liq. ammon. fort. 2 ccm., water 8 ccm. Drive off excess of ammonia by heating in a glass vessel over a sand-bath. The solution is allowed to cool and is then mixed with 4–6 times its volume of strong alcohol. A bright-red precipitate is formed. This is filtered off, washed and dried. The powder keeps for months. [Bolles Lee.]

A freshly prepared aqueous solution of this powder is employed for staining.

The following solution of **Uranium-Carmine** is recommended for sections of tissues infiltrated by celloidin, as it leaves the celloidin uncoloured:—

Sodic carminate	grm. i.
Uranium nitrate	grm. $\frac{1}{2}$.

Rub up together. Add distilled water 100 ccm. and boil for half-an-hour. Filter when cool.

Chrome-hardening is necessary, but after-hardening

in alcohol is permissible. Sections are left 15–20 minutes in the stain, but overstaining does not occur in twenty-four hours. Treat subsequently as usual.

With ammonia-carmine structures are stained of a rose tint, or in different shades of red; nuclei are most deeply stained, cell-substance less, and ground-substance least of all.

Carmine Combinations.

Picro-carmine (Ranvier).

Carmine	1 grm.
Liq. ammon. fort.	3 ccm.
Water	10 ccm.

Rub the carmine up with the water, add the ammonia to the fluid in a test tube; aid solution by gentle heat. When dissolved and the solution cold, pour it into 200 ccm. of cold sat. aq. sol. picric acid. Place the solution in an open vessel, and with gentle warmth evaporate to one-third of its bulk. Filter.

Hoyer states that a combination having all the advantages of picro-carmine, "without any of its disadvantages," is obtained by dissolving his carmine powder in a concentrated solution of neutral picrate of ammonia.

Soda-Picro-Carmine (Löwenthal).—Dissolve 1 grm. caustic soda in 100 ccm. water; add 0·4 grm. carmine. Boil the mixture for 10–15 minutes, and add 100 ccm. water. Add sufficient 1 per cent. aq. sol. picric acid to just dissolve the precipitate it first throws down. The fluid is allowed to stand 2–3 hours, and then filtered several times through the same filter paper. The solution is apt to grow turbid with lapse of time.

Stain sections for a few minutes to one hour in picro-carmine. Pour off excess of stain from the section as it lies on the slide; do not wash. Mount in glycerine, glycerine-jelly, or Farrant's solution, to which add 1–5 per cent. formic acid. This brightens the colour. Sections improve after keeping some time in the mounting medium.

Sections may also be mounted as usual in balsam if the precaution be taken to add to the water and alcohol used in washing some solid picric acid, which dissolves readily in these media.

In a successful picro-carmine preparation the nerve-cells are pink, nerve-cell nuclei deep red, connective-tissue nuclei and nuclei of vessels rose, axis-cylinders red, and myelin is yellow.

Borax-Carmine (aqueous solution).

Carmine	0 : 5 grm.
Borax	2 grm.
Aq. dest.	100 ccm.

Mix together in a porcelain dish and heat to boiling. Add dilute acetic acid (5 per cent.) till the colour becomes rather like that of ammonia-carmine. Allow to stand 24 hours. Filter.

Borax-Carmine (alcoholic solution—Grenacher).

Carmine	2–3 grm.
Borax	4 grm.
Aq. dest.	93 ccm.

Dissolve slowly by allowing the mixture to stand, with occasional stirring, 2–3 days. Then add an equal

vol. of alcohol 70 per cent. Allow to stand 36 hours. Filter.

Leave sections in either of these fluids ¼ hour to several hours; no fixed time can be stated. [Overstaining practically impossible.] Then wash in acid-alcohol (HCl. *fort.*, 1 ccm., alcohol 70 per cent., 100 ccm.) for about one minute; this brightens the specimen and renders the staining more selective. Wash in water; alcohol, clove oil, balsam, as usual.

Neutral Borax - Carmine (Nikiforow). — Boil together carmine 3 grm., borax 5 grm., and water 100 ccm. Add enough ammonia to dissolve the carmine. Evaporate down to rather less than one-half the original volume. Add dilute acetic acid until the cherry-red colour changes distinctly; avoid adding excess (if desirable, neutralize with ammonia). A powerful direct nuclear stain.

With borax-carmine structures are stained in shades of red—the nuclei deepest, cell-bodies lighter, ground substance but faintly stained.

Very suitable for staining in the mass.

Alum-Carmine (Grenacher).—Add ½–1 grm. carmine to 100 ccm. of 1–5 per cent. sol. of common or ammonia-alum; boil for 20 minutes. When cool, filter.

Stain two hours or more. Overstaining scarcely possible. Wash in water; dehydrate, clear, mount, as usual.

According to Upson, sections stain in a few minutes if to every 5 ccm. of the alum-carmine 1–3 drops of phosphomolybdanic acid are added.

Alum-carmine stains nuclei a violet red, cell-protoplasm a lighter red, ground substance very faintly.

Haug has lately recommended the following stain as giving a particularly sharp and clear representation :—

Carmine	1 grm.
Borax	1 grm.
Ammonia-alum	2 grm.

Rub up in a mortar and boil for half-an-hour or so with liq. aluminis acetici, 100 ccm. Decant. Filter in twenty-four hours. Use in a few weeks. Stain slowly. Wash in water, and proceed as usual. The fluid retains its qualities for long.

Lithium-Carmine (Orth).

Carmine	$2\frac{1}{2}$ grm
Sat. aq. sol. lithium carbonate . .	100 ccm.

The carmine is simply dissolved in the lithium solution. Stain as in the case of borax-carmine. Subsequent treatment (acid-alcohol, etc.) is also the same.

Nuclei are stained deep red, cell-bodies a lighter red, and ground substance is only faintly stained. A very good nuclear dye, usually successful even with specimens difficult to stain. With such, if necessary, the proportion of carmine may be increased to 5 per cent.

Picro-Lithium-Carmine (Orth).

The lithium-carmine sol. given above	1 part.
Sat. aq. sol. picric acid	2–3 parts.

Mix together. If the resulting stain should be too red or too yellow a little of the other colour should be added.

Stain 6–12 hours, longer if necessary. Proceed as

with lithium-carmine. The results are the same, except that the picric acid stains the myelin of the nerve-tubes yellow.

For preparations difficult to stain the following (Haug's formula) may be recommended. The solution can be used directly after preparation. It is very durable:—

Carmine	1 grm.
Ammon. Chlorat.	2 grm.

Rub up in a mortar. Boil in 100 ccm. water. The fluid is cloudy. After cooling add by drops 15–20 ccm. liq. ammon. caust., and 0·3–0·5 grm. lithium-carbonate. The cloudiness disappears. Filter.

Sections stain rapidly in a few minutes. Differentiate over-stained sections in acid-alcohol (*see* Borax-Carmine) Wash well in absolute alcohol. Proceed as usual.

Nuclear Stains.

Hæmatoxylin.—Solutions kept for any length of time decompose. An exception must be made in the case of Ehrlich's hæmatoxylin, which remains unaltered and retains its staining power unimpaired for years. Hæmatoxylin stains should be allowed to ripen for a few weeks, at any rate, before use.

Hæmatoxylin solutions easily overstain. Overstaining is corrected by certain measures presently to be mentioned.

Many formulæ are in use, of which the following may be given:—

Hæmatoxylin (Böhmer).

(i.)	Hæmatoxylin crist.	1 part.
	Alcohol	12 parts.
(ii.)	Alum	1 part.
	Water	320 parts.

These two solutions may be kept in stock. Some days before staining, add (i.) to (ii.) until a violet colour appears (a few drops only necessary). The solution is then allowed to ripen a few days.

Wash chrome-hardened sections well before placing them in a hæmatoxylin stain. It is not advisable to pass them from alcohol into the stain, as a deposit is apt to form. Wash in distilled water, or in 1–2 per cent. alum, aq. solution beforehand.

A section should be removed from Böhmer's stain from time to time (after the lapse of a few minutes, to begin with) to see if staining is sufficient. It may be examined under a low power to judge of the appearance of the nuclei—in the earlier attempts. The section should not be too deep blue. If overstained, place in $\frac{1}{2}$–1 per cent. alum aq. sol. for from one to several hours. After this treatment sections should be thoroughly well washed in distilled water. Dilute HCl. may also be used to remove excess of stain (HCl. *fort.*, 1 drop; water, 40 ccm.); in this the section acquires a faint violet tinge. After a few seconds remove to following solution :—strong ammonia, 1 drop; water, 100 ccm. A brief stay in this neutralizes the acid. Wash in water, and proceed as usual. The same measures hold good in the case of overstaining with other hæmatoxylin solutions.

If the section is properly stained, wash directly on

removal from the stain in ordinary water; dehydrate, clear, mount, as usual. (It is said that clove-oil is prejudicial to the permanence of the hæmatoxylin stain. If it is used in clearing, wash away by toluol or other clearing agent indifferent to the dye. Or use creosote or origanum instead.)

Hæmatoxylin (Delafield).—Take of sat. aq. sol. ammonia-alum, 400 ccm. Add hæmatoxylin crist., 4 gram., dissolved in 25 ccm. of alcohol. Expose to light and air in an unstoppered bottle for 3–4 days; filter. Add glycerine and methyl-alcohol, each 100 ccm. Allow the solution to stand until the colour is dark; filter, and keep in stoppered bottle. Allow to ripen a couple of months before use.

For staining, add water to a small quantity of the dye until a dilute stain is obtained. Proceed as with Böhmer's stain.

Acid Hæmatoxylin (Ehrlich.)

Dist. water	100 ccm.
Abs. alcohol	100 ccm.
Glycerine	100 ccm.
Glacial acetic acid	10 ccm.
Hæmatoxylin.	2 gram.
Alum in excess.	

Expose to light for 2–3 weeks; the colour darkens. Keep in a stoppered bottle. The dye remains efficient for years.

Stain sections for 5–10 minutes; occasionally rather longer is necessary. Wash in ordinary water; here the section turns blue. Dehydrate, clear, mount, as usual.

In the case of this solution decomposition from precipitation of certain constituents (such as occurs with ordinary alum-hæmatoxylin solutions) is prevented by the addition of the acid.

Haug has lately recommended the following solution as especially useful for preparations difficult to stain, owing to the method of hardening, or other cause. It stains rapidly:—

Hæmatoxylin extr.	1 grm.
Dissolve in abs. alcohol.	10 ccm.

Add above solution to 200 ccm. liq. aluminis acetici. At first the fluid is a deep violet, but in the course of some weeks the colour ripens to a brown-black. To hasten ripening add a few ccm. sat. aq. sol. lithium carbonate. The solution remains efficacious for long.

Stain sections thoroughly, and then remove excess of colour by rapid use of acid-alcohol. [*See* Borax-Carmine.] Wash well in water to remove the acid; the sections turn blue. Contrast—staining, if desired, by means of eosin, and especially with aq. sol. erythrosin. Wash, dehydrate, clear, mount, as usual.

Hæmatoxylin stains in shades of blue (occasionally red). All nuclei present are deeply stained, cell-bodies more lightly.

Cochineal. — The following formulæ give good results:—

Alum-Cochineal (Czokor).

Cochineal	1 gram.
Alum	1 gram.

are rubbed up together in a mortar and dissolved in

100 ccm. distilled water. Boil down to one-half the volume. When cool filter several times.

Stain for twenty-four hours and more; overstaining is practically impossible. Wash in water, dehydrate, clear, mount, as usual. Nuclei have a violet, cell-bodies and processes a reddish tint. Ground substance very lightly stained. (A very precise stain.)

Cochineal (Meyer; quoted by Bolles Lee). — Macerate cochineal in coarse powder for several days in 70 per. cent alcohol, taking 1 gram. cochineal to 10 ccm. alcohol. Stir often. Filter before use.

Before placing a section in the stain pass it rapidly through alcohol 70 per cent., and dilute the stain if necessary with alcohol of the same strength; also wash afterwards in the same. Other fluids cause precipitates. Wash in the alcohol till no more colour comes away. Overstaining may be corrected by momentary use of acid-alcohol. Sections may be stained in a quarter of an hour or so; or longer in dilute solutions. After washing in the alcohol, as advised, dehydrate, and proceed as usual.

Structures are stained red, the cell-nuclei most deeply. The ground substance is more stained than in the case of alum-cochineal—in other words, the stain is more diffuse.

The *Carmine combinations* already gives (Picro-, Borax-, Lithium-, Alum-Carmine), may be again alluded to here as nuclear dyes.

Several of the *anilin dyes* may be mentioned as nuclear stains. At any rate, they act as such when used upon chrome-sections. They cannot be relied on for staining the protoplasm of the nerve-cell, as in the processes of washing out and dehydration a variable

amount of the colour is extracted from that substance. Nuclei of nerve-cells and of connective-tissue are stained by them, especially the latter. When employed upon sections from tissues hardened in alcohol some of these anilins stain the cell-protoplasm effectually. [*See* "Special Stains."]

Amongst those which might be placed with the Nuclear Stains are Bismarck-brown, Gentian-, and Methyl-violet, Victoria-blue, Dahlia, Methylene-blue, and Toluidin-blue. [For methods of use, *see* "Anilin Stains."]

Stains for the Medullated Nerve-Fibres.

Weigert's Method.—Harden tissues in potass. bichromate solution or in Müller's or Erlicki's fluids, finishing, if desired, in alcohol. [*See* "Hardening Methods."] Infiltrate with celloidin and fix on cork in the usual way. [This is of course unnecessary if other imbedding media are used.] The block is now placed for a day or two in sat. sol. neutral acetate of copper diluted with an equal volume of water—this solution is kept at about 37 degrees C. The tissues become green. The preparation may then be kept till required in alcohol 80 per cent. Sections, cut under alcohol, are placed in the following stain :—

Hæmatoxylin	1 grm.
Alcohol	10 ccm.
Water	90 ccm.

which is ready for use in 1–2 weeks. At the time of use add sat. sol. lithium carbonate, a few drops to a watch-

glassful of stain. Sections remain a varying time in the stain, dependent on the nature of the tissue. About 2–4 hours for the medulla of brain, 24–36 hours for the intra-cortical meshwork, Staining is expedited by the incubator-temperature (37·5 degrees C.)

On removing sections from the stain, rinse with water and place in following solution :—

Borax	2 grm.
Ferricyanide of Potassium	2·5 grm.
Water	200 ccm.

Here sections remain until complete differentiation of the grey and white matter is obtained. This part of the process requires supervision. Remove from the fluid when the grey matter becomes a light brown and the white a deep violet. If thick sections do not become decolourised properly in this solution they may be transferred for twenty-four hours to alcohol and then replaced in the ferricyanide. To avoid excessive decolourisation it is as well to subject sections to the action of the differentiating fluid for varying lengths of time. [The same holds good for all modifications of Weigert's process.]

When properly differentiated, wash the sections, dehydrate in alcohol 96 per cent., and then merely momentarily in abs. alcohol (to preserve the celloidin intact), clear in origanum oil or xylol, preferably the latter, mount in xylol-balsam.

Double-staining (picro-carmine or alum-carmine suitable for the purpose) may be carried out after the final washing, if it is desired to show nuclei and cell-bodies. But such contrast-staining had better be avoided when the object is to show the finest nerve-fibres.

Medullated nerve-fibres appear blue-black against a brownish-yellow background.

The treatment with copper-solution may be deferred until sections have been made, individual sections being placed in the solution. This plan has the advantage that other stains, to the use of which copper is prejudicial, may be used in addition to hæmatoxylin.

Recently *Berkley* has stated that the best results, as regards staining fine nerve-fibres, are obtained by the copper-hæmatoxylin process when the tissues have been hardened in Flemming's fluid [osmic acid 1 per cent., 10 ccm.; glacial acetic acid 1 per cent., 10 ccm.; chromic acid 1 per cent., 25 ccm.; water, 55 ccm.] Pieces 3–5 mm. thick and of moderate size are kept in the above for thirty hours at 25 degrees C. Transfer without washing to absolute alcohol for twenty-four hours. Change twice. Imbed in celloidin or not. Sections are washed in water and placed in the acetate of copper solution for a night. Wash rapidly in water, and place in a hæmatoxylin solution made as follows:—Boil 50 ccm. dist. water well in a flask for some minutes, add 2 ccm. sat. sol. lith. carbonate, and continue boiling for a minute or so. Add 1·5–2 ccm. of a 10 per cent. sol. of hæmatoxylin in abs. alc. Shake up. Cork, and allow to cool.

In this solution sections remain 15–20 minutes at 40 degrees C. On cooling, wash in water, and treat with borax-ferricyanide, and as usual after Weigert.

Weigert's Earlier Method, which some consider possesses certain advantages over the newer one, is as follows:—The tissues are hardened as before and imbedded in celloidin. Sections are cut into alcohol From this they are passed into the hæmatoxylin solution

(kept at 35–40 degrees C.). Subsequent treatment, as in the method first mentioned.

The finest nerve-fibres, however, are better brought out by the copper method.

Staining "in toto" with Weigert's Earlier Method.—Beevor* got good results ("staining of the finest fibres generally") in the case of the marmoset's brain by proceeding as follows:—The brain had already been in methylated alcohol for 2–3 weeks by a mistake, when it was put in pot. bichrom. sol. 3 per cent. After a month in this it was cut vertically and laterally into pieces about one-eighth inch thick, or less, washed in methylated alcohol for 1–2 days, and then put into Weigert's hæmatoxylin for four days, the fluid being kept at 40–50 degrees C. for 3–4 hours each day. The stain was about double the strength of the original, thus—hæmatoxylin 2 grm., abs. alc. 14 ccm., water 130 ccm. The pieces of brain were then slightly washed in water and put into borax-ferricyanide sol. (water in the prop. of 100 ccm.); this was changed until no more brown colour came away, and the pieces were again washed in water. Subsequently they were passed through alcohol, oil of cloves, and oil of turpentine to paraffin. Sections were then cut and mounted, the paraffin being removed by xylol.

With specimens hardened in bichromate and afterwards in alcohol—in the usual way—Beevor has not had good results. Weigert also obtained imperfect results with his method (presumably the earlier one) as applied to pieces in bulk. The newer method—

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* *Brain*, vol. viii, p. 239.

with copper acetate—does not appear to have been applied in this way in the case of brain-tissue.

In addition to the method suggested by Berkley, a great number of modifications of Weigert's process have been introduced, some of which—those regarded as important—are subjoined.

Weigert himself has modified his method in such a manner that differentiation as performed in the original method is dispensed with. Harden as usual, and imbed in celloidin. Float the imbedded pieces for twenty-four hours in the incubator in the following solution:—

Neutral acetate of copper, sat. sol. in the cold, filtered . .	equal parts.
Tartrate of soda solution 10 per cent.	

Then keep for 24–48 hours (latter for large pieces) in simple aq. sol. neutral acetate of copper in the incubator. Wash lightly in water; 80 per cent. alcohol; cut. Have ready Sol. A:—

Sat. aq. sol. lithium carbonate . .	7 parts.
Water	93 parts.

and Sol. B:—

Hæmatoxylin	1 part.
Abs. alcohol	10 parts.

Just before use mix 9 parts of A with 1 part of B. Leave sections in the mixture 4–5 hours (twenty-four hours are not harmful). Wash in water, then in alcohol 90 per cent., clear in anilin-xylol [2 parts

anilin-oil, 1 part xylol], to be removed by pure xylol. Mount in xylol-balsam.

Black staining (of medullated fibres) on a clear red ground. Over-stained sections are treated by borax-ferricyanide, as usual.

Paneth first suggested that the stain should be made with extract of logwood, which is much cheaper than pure hæmatoxylin. Make following solution:—

Commercial extract of logwood . .	1 grm.
Abs. alcohol	10 ccm.
Water.	90 ccm.

Filter. Add to the solution 8 drops conc. aq. sol. lithium carbonate. Sections — prepared after the original method—require 18–24 hours to stain in the above at the ordinary temperature of the room. After staining, proceed as in the original method.

Kaiser has quite lately introduced the following rapid process, essentially differing from Weigert's method in the use of Marchi's fluid in hardening and of sesquichloride of iron as a mordant, in place of copper acetate:—Small pieces of tissue are placed in Müller for three days; they are then cut into sections of 1–2 mm. thickness and replaced in Müller for another six days. Transfer to Marchi's fluid [Müller 2 parts, osmic acid 1 per cent. sol. 1 part]; here the sections lie for eight days. Wash, after-harden in alcohol, infiltrate with celloidin. Cut sections. These are treated for about 5 minutes with the following solution:—

Liq. Ferri sesquichlor.	1 part.
Aq. dest.	1 part.
Spirit rectific.	3 parts.

Wash in Weigert's hæmatoxylin solution, and heat in a fresh quantity of the same for a few minutes. The fluid must not be heated to boiling, on account of the celloidin. Wash sections in water and differentiate as in Pal's method (*v. infra*). Transfer momentarily to water containing some ammonia, in order to neutralise the oxalic acid. Wash, dehydrate, clear in xylol, mount in xylol-balsam.

Medullated nerve-fibres appear dark brown or deep black. This method gives satisfactory results, but more extensive experience is needed to determine whether these are consistently obtained.

Pal's modification of Weigert's process is widely employed. The Weigert-Pal method is as follows:— Tissues are hardened in chrome-salts as in Weigert's method; the copper treatment is omitted. Sections are cut as usual. If these do not appear to contain sufficient chrome (have, that is, a greenish tint), they should be placed in pot. bichrom., 2–3 per cent. sol. for twenty-four hours or so. After washing away excess of chrome, transfer the sections to Weigert's hæmatoxylin solution, to which lith. carbonate is added, as usual. Here they remain 24–48 hours. Wash in water; the sections should be a very deep blue—almost black; if they are not, add to the water lith. carbonate sat. sol., in the proportion of about 4 per cent. Transfer to fresh aq. sol. permanganate of potash, $\frac{1}{4}$ per cent., where they remain 20–30 seconds. Remove to the following differentiating solution:—

Oxalic acid pur.	1 grm.
Sulphite of potassium	1 grm.
Aq. dest.	200 ccm.

In a few seconds differentiation is complete; the grey matter is decolourised, the white matter has a blue-black tint. If this result is delayed, sections may be put back into the permanganate a few seconds, and then replaced in the different. fluid. Move them about in the latter. From this they are transferred to distilled water and washed well. Exposure to a strong solution of lith. carbonate (5–30 minutes) is now advantageous. The staining is deepened and rendered more precise. Wash again; double-stain with alum-, borax-, or picro-carmine; wash, dehydrate, clear in xylol; mount in xylol-balsam.

Nerve-fibres black or blue-black; all other structures decolourised.

The sections are better suited for double-staining than those obtained by Weigert's method, as the non-medullated tissues are quite decolourised. This advantage, however, is not secured without additional risk; unless considerable care is exercised differentiation will be carried too far, and the finest intracortical and tangential fibres decolourised.

The lithium-carbonate solution, usually added to the hæmatoxylin stain in these processes, need not be employed until after the latter has acted. In this case sections are removed from the hæmatoxylin after the lapse of the usual time, and placed in sat. aq. sol. lith. carbonate (or in a 2–4 per cent. sol., used for a longer period), until they are sufficiently dark. Proceed then as usual. By following this plan the same hæmatoxylin solution may be repeatedly used. Possibly the hæmatoxylin-lithium sol. may be employed several times over. But the fact that it is quite opaque renders it much less convenient to work with than the

translucent plain solution, even if the supposition be correct.

Lissauer suggested the following rapid method of conducting Weigert's process; the writer has obtained good results by it:—Sections as thin as possible are cut from the organ hardened in Müller's fluid, and are placed in a 1 per cent. sol. of chromic acid, which is then heated until bubbles begin to form on the surface. Wash lightly in water, and heat similarly in Weigert's hæmatoxylin solution until bubbles form. Treat with pot. permang. sol. and the differentiating fluid, as in Pal's process.

Schaefer's modification of Pal's process is to be strongly recommended. Pieces hardened in Müller, and afterwards in alcohol [no intermediate washing], are imbedded in celloidin, and sections cut. These are placed in water, and from this transferred to Marchi's fluid [*see* "Hardening Methods"], in which they are left a few hours. Wash again in water, and transfer to acetic-acid-hæmatoxylin, made by dissolving 1 grm. hæmatoxylin pur. in a little alcohol, and adding to the solution 100 ccm. of 2 per cent. sol. acetic acid. Leave sections in this overnight. On removal, they are quite black. Wash in water, and pass through pot. permang. sol., Pal's bleaching fluid, &c—as in the original process.

Kultschitzky's Stain for medullated nerve-fibres is also a good one. Tissues are hardened in Müller, or better, in Erlitzky's fluid, washed in water to remove excess of chrome, and sections cut as usual. K. especially insists upon the fact that tissues can be washed after the hardening in Erlitzky without detriment. Thus excess of the hardening reagent is removed.

This is an advantage over Weigert's process. The sections are placed in the following stain for 18–24 hours :—

Sat. aq. sol. boracic acid	20 ccm.
Aq. dest.	80 ccm.

to which is added hæmatoxylin, 1 grm., dissolved in abs. alc. q.s. This solution is yellow when first made, but in 2–3 weeks it becomes red, and may then be used. Before use, acidify by adding to a watch-glassful of stain 2–3 drops acetic acid. More recently the following simpler formula has been introduced. It is quite as efficient :—

Hæmatoxylin	grm. i.
Abs. alcohol	q.s. ad. solv.

Add this solution to 100 ccm. of a 2 per cent. aq. sol. acetic acid. Stain for twenty-four hours. After staining, place sections in following solution for 2–3 hours, for differentiation :—

Sat. aq. sol. lithium-carbonate . .	100 ccm.
Ferricyanide of potassium, 1 per cent. aq. sol.	10 ccm.

Wash well in water, then in alcohol. Xylol, xylol-balsam.

The medullated fibres appear deep blue, other tissues faint yellow.

Wolters's Method (sometimes referred to as the Kultschitzky-Wolters Method) is now largely used, and highly recommended. It shows the finest nerve-

fibres—amongst them the tangential fibres of the outermost layer of cortex—well.

Harden in Müller, wash in alcohol. Cut in celloidin. Place sections in Kultschitzky's hæmatoxylin.

Hæmatoxylin (Grübler)	2 grm.
Abs. alcohol	q.s. ad. solv.
Acetic acid, 2 per cent. aq. sol. . .	100 ccm.

Here sections remain twenty-four hours in a paraffin oven at 45 degrees C. At the end of that time they are dipped in Müller, and then differentiated after Pal [pot. permang. sol., followed by the sulphurous acid]. Wash in water. Dehydrate, clear in xylol, xylol-balsam.

An intense myelin stain is obtained. Nerve-fibres are blue-black; ganglion-cells yellow, or yellow-brown.

Kaes gets even better results with the above method, by substituting Flemming's mixture for Müller. [Compare Berkley's method of conducting Weigert's process.] The tangential fibres then show particularly well. To retain with certainty the finest fibres, differentiate only so far that the grey matter has a yellow tint.

Exner's Method. — For description of this, *see* "Hardening Methods."

The Pal-Exner Method (which is said to furnish permanent preparations) is as follows :—Small pieces of quite fresh brain are hardened for 4–6 days in ten times the bulk of osmic acid, 1 per cent. Fresh solution on the second day. Wash well in water, and then in absolute alcohol, two minutes. Infiltrate with celloidin or paraffin in the usual way. Cut sections into glycerine thinned with water (3 glycerine, 1 water). Wash away the glycerine with water, and differentiate

after Pal's method [10–15 seconds in pot. permang. sol. Stop the sulphurous acid action when the medulla is black and cortex deep grey]. Wash well. After-stain with Magdala-red or picro-carmine. Dehydrate, clear, mount, as usual.

Anilin Stains.*

Out of a large number of anilin dyes employed by the writer upon hardened sections of brain, it is sufficient to give the following, as most of these dyes resemble each other in action when used, at any rate, in the manner described below. As will be seen, they are chiefly nuclear stains.

In each case the action of the stain was examined upon sections from tissues hardened in chrome-salts, with short after-hardening in alcohol (the plan followed with the stains already given), and upon others from tissues fixed in sublimate, followed by alcohol. [*See* "Fixation Methods."] As a general rule, sublimate sections stained very much more quickly than chrome ones.

It should be mentioned that comparatively recently important results have been obtained with anilin dyes upon tissues hardened in alcohol. [*See* methods of Nissl and Rehm—described later.]

The results with the single stains are first given, then those obtained with some anilin combinations. Chrome sections are first referred to, unless otherwise stated.

Anilin Blue-black.—The English preparation should

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* The most important should be kept in stock. *See* the catalogue of Grübler or Münder.

be employed, in aqueous solution $\frac{1}{4}$ per cent. Stain for $\frac{3}{4}$–1 hour. Overstaining should be avoided, as the dye can only be extracted to a slight extent. Wash, dehydrate in alcohol, clear with creosote or other agent excepting clove-oil (which alters the colour in time), mount in balsam.

With sections difficult to stain, an alcoholic solution [$\frac{1}{6}$–$\frac{1}{3}$ per cent., made with abs. alc., or good methylated spirit] may be employed. It will stain such sections when the aq. sol. fails, or acts exceedingly slowly. The alcoholic solution also stains celloidin less deeply.

For the cerebellar cortex, Bevan Lewis recommends a modified proceeding, as follows:—After staining with the aqueous solution and washing in water, as usual, the section is immersed for 20–30 minutes in a solution of chloral hydrate, 2 per cent. Then transfer to the following:—

Solution of chloral (2 per cent.) } āā
Oil of cloves . . . . . }
Alcohol q.s. to dissolve and make a clear solution.

Add the alcohol by degrees, stirring, and avoiding excess. During use, cover the watch-glass containing the solution to prevent evaporation. The chloral removes diffuse staining whilst the clove-oil clears the section. Examine occasionally under a low power, and when a satisfactory stain is obtained, wash the section with absolute alcohol; clear with clove-oil, and mount in balsam.

Anilin blue-black stains the nerve-cells with their nuclei and processes, axis-cylinders, connective-tissue nuclei, and those of vessels. The tint varies from blue-grey to deep blue-black. Nerve-cell nuclei and axis-

cylinders are stained deepest; connective-tissue structures are not well stained. The basis-substance of the cortex is pale grey.

With sublimate sections a diffuse stain results very quickly. The dye cannot be recommended for these.

This excellent stain has long been employed in this country, and is now used more extensively than formerly on the Continent, owing, doubtless, to the recognition of the value of the English preparation.

Indulin.—This substance (as supplied by Grübler), gives results very like those obtained with English anilin blue-black; the stain is, perhaps, rather more blue. It may be used in precisely the same manner as the last.

Nigrosin (Grübler).—The same remarks apply in this case, both as regards the method of use and the results. The latter, however, appear inferior to those obtained with the English blue-black. [Nigrosin is much used abroad.]

Anilin-Blue.—Use quite a dilute aqueous solution (translucent in a thin layer on a slide). Stains in 5–10 minutes (very rapid). Proceed as with anilin blue-black. Like that dye, and unlike most anilins, anilin-blue does not wash out in alcohol. It resembles blue-black in its affinity for nerve-cells, as opposed to connective-tissue elements, but is inferior in being more diffuse in action.

With sublimate sections the staining is far too diffuse, even in a few minutes.

Toluidin-Blue.—Employ an aqueous solution 0·25 per cent.; the powder dissolves better if a little alcohol is added. Stain for 24–48 hours. Sublimate sections

particularly suitable for this dye; they stain in half-an-hour, but the longer period is advisable. The cortex appears a uniform deep blue, the medulla a fainter blue. Excess of stain is removed by water and then by alcohol (first methyl. spirit, then absolute.) The colour comes away in clouds. Presently these cease to form; the section is then at once transferred to xylol. Further extraction is thereby prevented; if the last alcohol used was absolute the xylol clears the section immediately. Mount in xylol-balsam.

The section has a light blue or purplish tint. Connective-tissue nuclei, including those of the vessel walls, are exceedingly well stained, also the nuclei of nerve-cells. These structures are deep blue or purple. Nerve-cells of similar though lighter tint. Their processes are stained about as well as in the case of chrome-hardened specimens treated by the ordinary dyes. The neuroglia basis is practically colourless. The stain is chiefly a nuclear one. Very good results are obtained with care.

With chrome-sections the results are not nearly so good. There is more diffuse staining; the sharpness of outline is absent.

Victoria-Blue. — Use sublimate sections. Stain about forty-eight hours in a deep blue aq. sol. Sections are now uniform deep blue. Proceed as in the case of toluidin-blue. Results are in general the same, but the body of the nerve-cell—outside the nucleus—is more easily decolourised than in that case, and consequently the staining of the cell-body is capricious. The removal from alcohol to xylol is advisable before the formation of colour-clouds entirely ceases. Consequently the neuroglia is not so well

decolourised in this case as in the last. Chiefly a nuclear stain.

With chrome the results are not so good; staining is more diffuse.

Safranin.—Obtain the most suitable variety for staining nuclei. Make a deep red aqueous sol. (by heat if necessary), or dissolve in equal parts of alcohol and water. Stain for 12–24 hours or more. Treat as in the case of Victoria-blue. Acid-alcohol [HCl. *fort.*, 1 ccm.; alcohol, 70 per cent., 100 ccm.; a few drops to a watch-glassful of alcohol], is often necessary in decolourisation. If used, wash well subsequently in absolute alcohol.

It is especially adapted for staining nuclei generally. [*See* "Methods of Staining after Fixation."] Much used in the study of nuclear figures. Commonly employed upon tissues fixed in chrome-aceto-osmic mixture.

After sublimate safranin is too diffuse. The results are not good.

Dahlia.—Stain sublimate sections for about forty-eight hours in a deep red aq. sol. Treat the over-stained section as in the case of Victoria-blue. The results are similar, except that the staining is red. Chiefly a nuclear stain.

With chrome sections the results are practically the same.

Gentian-Violet.—Stain sublimate sections for about forty-eight hours in a deep violet aq. sol. Proceed as with Victoria-blue. Acid-alcohol may also be necessary. The results are similar as regards the parts stained, but the tint is violet. Fickle staining of the body of the nerve-cell. A nuclear stain.

Much the same results with chrome sections.

Methyl-Violet.—Stain sublimate sections for 2–3 days in a deep violet sol. [alcohol + water, āā]. Proceed as with Victoria-blue. Acid-alcohol may be necessary. The same parts stained—of a violet colour. A nuclear stain.

With chrome sections the results are somewhat inferior.

Methylene-Blue.—Stain forty-eight hours in a deep blue aq. sol. Proceed as with Victoria-blue. Results similar, except that the blue is of a different shade, and nerve-cell nuclei do not stain so well. Chiefly a stain for connective-tissue nuclei.

With sublimate sections much the same results.

Bismarck-Brown.—Stain from one to several hours in a rather deep brown solution [alcohol + water, āā]. Proceed as with Victoria-blue. Excess of stain is not so easily removed as in some cases. Connective-tissue nuclei stain very well, including those of vessel-walls (deep brown). Nerve-cells indifferently.

With sublimate sections the staining is more diffuse.

Congo-Red.—Stain 12–18 hours in a deep red sol [alcohol + water, āā]. Sections deep red-brown. Wash in water and in alcohol. In the latter the colour comes out to some extent. If the section is now placed in acid-alcohol [HCl. *fort.*, 1 ccm.; alcohol, 70 per cent., 100 ccm.] the colour turns to a deep brown-black, or purple. Leave some hours in the acid-alcohol. Wash, dehydrate *rapidly*, clear in xylol; xylol-balsam. Axis-cylinders in transverse and longitudinal section are stained brown-black. Nerve and connective-tissue cells of the same tint, or purplish.

Ground-substance is light brown. The axis-cylinders are particularly well stained in this way, hence the method is especially applicable to transverse sections of pons, medulla and cord.

The writer lit upon this process accidentally whilst examining the action of anilin dyes. The same observation was made by Nissl (1886). His procedure is as follows:—Bichromate sections are passed through alcohol 95 per cent. into aq. sol. Congo-red, 5 : 400, where they remain seventy-two hours. Transfer to alcohol, 95 per cent., for 5–10 minutes, then to nitric-acid-alcohol (HNO_3, 3 ccm.; alcohol, 100 ccm.) for six hours. Alcohol, clove-oil, balsam.

With sublimate sections the result is not good, a diffuse stain being obtained.

Methyl-Green.—This dissolves out with such facility in water and alcohol that it appears to be of little use for hardened tissues. From an abstract of the original account it seems that Erlitzky adopted methyl-green for staining tissues hardened in his fluid. [*See* "Hardening Methods."] An aqueous or alcoholic solution, 1·5–2 per cent. was used, the former preferably. Sections were stained 12–24 hours. It was found to be a nuclear stain, especially bringing out the nuclei of connective-tissue and blood-vessels.

For staining nuclei in fresh tissues methyl-green is well known to be most suitable. For this purpose it is commonly employed as a strong aqueous solution containing about 1 per cent. of acetic acid. Wash out with water slightly acidulated.

Certain anilin dyes make excellent *contrast stains*. In this capacity they are used to stain the ground substance of the cortex. [*See* "Combination Stains."]

Eosin, Bengal rose, benzo-purpurin, and picric acid are examples.

Anilin Combinations.—These are numerous. It is sufficient to mention some of the best, with the methods of use.

Safranin and Anilin-Blue (Garbini).

(i.)	Anilin-blue (soluble in water)	1 grm.
	Abs. alcohol	1–2 ccm.
	Dist. water	100 ccm.
(ii.)	Safranin	1 grm.
	Alcohol	100 ccm.
	Water	200 ccm.

Chrome-hardened sections lie for about four minutes in (i.), are then washed in water, and placed in ammonia sol. (1 per cent.) until the colour has nearly disappeared. Treat 5–10 minutes (until the colour has returned) with HCl. sol. 0·5 per cent. Wash in water and stain in (ii.) 5–10 minutes. Wash. Dehydrate in alcohol; xylol, xylol-balsam.

Nerve-cells (extra nuclear parts) deep blue, cell-nucleus reddish-blue (occasionally red, occasionally deep blue), connective-tissue and vascular nuclei deep rose, ground-work of cortex pale blue, medulla reddish-yellow, including the medullary rays. Affords a very good differentiation.

Biondi-Ehrlich Stain (Methyl-green, acid-fuchsin, and orange).—Directions are often given for making this, but the writer recommends its purchase from the stain agents. It is generally employed upon sublimate sections, which remain in the stain 6–24 hours. Dehy-

drate and differentiate in alcohol; xylol, xylol-balsam. The sections appear light purple.

Nerve-cells light violet, their nuclei deep violet, connective-tissue nuclei green-blue, blood-vessels deep red [under a low power as if injected], owing to staining of the blood corpuscles, ground-work of cortex light red. With chrome sections the results are the same, except that the blood-vessels are not brought out as in the former case.

The following may be mentioned in addition :—

Gentian-Violet and Eosin.—Stain chrome sections in an alcoholic solution of gentian-violet diluted with one half its vol. of water. Wash out with alcohol, and acid-alcohol if further differentiation required. Stain for a minute or two in fairly strong aq. sol. eosin. Dehydrate rapidly in alcohol; xylol, xylol-balsam.

Other Combination Stains.

When it is desired to contrast certain tissue elements with others, combination stains are employed. No great experience is needed to show that ingenuity and time can be better employed than in increasing the number of these combinations, for they do not promise to promote our knowledge of cerebral structure materially.

The results now given apply to chrome sections [tissues hardened in chrome salts, washed, and after-hardened a day or two in alcohol].

Hæmatoxylin and Picric-Acid.—Stain in hæmatoxylin (Ehrlich's or other), about quarter of an hour if the former is used. Wash in ordinary water, expose a few minutes to alcohol rendered yellow by addition of

a few crystals of picric acid. The section changes from blue to a dirty yellow, and a brownish colour is discharged. Clear in xylol, mount in xylol-balsam.

Nerve-cells of a yellow tint, their nuclei a light violet; connective-tissue nuclei and nuclei of blood-vessels deep violet; ground substance yellow—a somewhat different tint to the nerve-cell, but not always properly differentiated from it.

Carmine and Picric Acid.—Stain in carmine, wash, treat as in last case. The section changes from red to reddish-yellow at the cortex; medulla yellow. Clear, mount, as before.

Nerve-cells pink, deepest at the nucleus. Ground substance of cortex a lighter pink. Connective-tissue nuclei are not well stained. [Better brought out by borax- or lithium-carmine.]

Hæmatoxylin and Eosin.—Stain in Ehrlich's hæmatoxylin, quarter-of-an-hour (or other formula may be used), wash in ordinary water. Stain in weak aq. sol. eosin a few minutes. The blue section changes to a reddish-violet. Wash, dehydrate in alcohol; xylol, xylol-balsam.

Nerve-cells deep pink, their nuclei light violet; connective-tissue nuclei and those of blood-vessels deep violet; ground substance light pink. A very good combination.

Hæmatoxylin and Benzo-Purpurin B.—Stain about ten minutes in Ehrlich's hæmatoxylin (or other may be used). Wash in ordinary water. Stain in weak aq. sol. benzo-purpurin B. for a few minutes. Wash; alcohol, xylol, xylol-balsam.

Nerve-cells a red-brick colour, their nuclei light violet; connective-tissue nuclei and those of blood-

vessels deep violet; ground substance reddish-violet. A very good combination.

Hæmatoxylin and Anilin Blue-Black.—Stain a few minutes in hæmatoxylin solution and then a few seconds in aq. sol. anilin blue-black 0·5 per cent. Wash; alcohol, creosote or xylol, balsam.

By this combination a very rapid staining of cell-elements is obtained. As anilin blue-black stains nerve-cells more especially and hæmatoxylin the connective-tissue cells, all the cells in a section are well shown. Especially suitable for cerebellar cortex, bring-out well the granule-layer and cells of Purkinje.

Hæmatoxylin and Safranin.—Stain very lightly in hæmatoxylin, wash in water, stain in safranin [safranin 1 grm., abs. alc. 100 ccm., water 200 ccm.]. Wash; alcohol, xylol, xylol-balsam.

Nerve-cells and processes light red, their nuclei violet; nuclei of connective-tissue and blood-vessels violet; ground substance faint red; medulla and medullary rays red; the latter are well shown. A good combination.

Anilin Blue-Black and Picro-Carmine.—Stain in picro-carmine $\frac{1}{4}$–$\frac{1}{2}$ hour; when deeply stained, place directly in the anilin solution ($\frac{1}{4}$ per cent. aqueous), merely draining off excess of picro-carmine. In about ten minutes the section acquires a deep violet tint—the action of the anilin is hastened by previous use of picro-carmine. Wash, dehydrate in alcohol, clear and mount.

Structures are stained in shades of violet. Nuclei of blood-vessels are especially well shown. A very good combination.

Picro-Carmine and Anilin-Green.—A good com-

bination for the cerebellum. Stain in picro-carmine $\frac{1}{4}$–$\frac{1}{2}$ hour in the incubator. Wash in water slightly acidulated with acetic acid, then quickly in distilled water. Transfer to aq. sol. anilin-green, about 1 : 1000. Stain for 12–24 hours. Wash in water, then in alcohol, till no further colour comes out; xylol, xylol-balsam.

Purkinje's corpuscles and the structures of the external layer of the cortex are pink, vessels also pink; corpuscles of granule-layer deep green (some pink); medullary cone green. On closer examination of last named, axis-cylinders are deep-green, myelin a light green. Especially good for low power view of cerebellar structure. The pink cortex with its prominent Purkinje-cells contrasts strongly with the deep green granule layer and light green medullary centre.

Carmine and Anilin-Blue (Recommended by Duval). —Stain with carmine, wash, stain 4–5 minutes in following solution:—Sat. alcoholic sol. anilin-blue 10 drops, abs. alc. 10 ccm. Clear in turpentine, without further treatment with alcohol. Mount in balsam. Sections are dark violet when removed from the anilin.

Nerve-cells reddish-violet (with axis-cylinders), blood-vessels bluish-violet, connective-tissue nuclei blue. A good combination.

Borax-Carmine and Picro-Carmine.—Sections may be placed first in one and then in the other, or the two stains combined. Add a few drops of picro-carmine to a watch-glassful of borax-carmine. Wash and dehydrate in alcohol rendered yellow by picric acid crystals; xylol, xylol-balsam.

Results much the same as those with carmine-picric acid, but nuclei are better stained.

Borax-Carmine and Indigo-Carmine.—Stain several

hours in Grenacher's alcoholic borax-carmine, place in acid-alcohol a few seconds, wash in water, then stain in a deep blue solution of indigo-carmine in alcohol (10–20 hours). Wash, dehydrate, clear, mount.

Nerve-cells are blue, their nuclei pink, connective-tissue and vascular nuclei red (occasionally violet). The ground-substance is green-blue (occasionally reddish-grey), medulla green.

Metallic Stains.

Much experimentation is now being carried on with metallic stains, and highly important results have already accrued from their use—especially in the case of nitrate of silver; and further developments may be looked for with much confidence. Especially in the case of persons dying insane is there a field for the employment of these stains, although it may be, in general, stated that the best results are scarcely to be expected with such tissues. These are obtained rather with the nervous-system of young animals and embryoes.

The following are the principal metallic stains employed, with the methods of use:—

Silver Nitrate (*Golgi-Cajal Method*).—This is especially adapted for embryonic brains and the brains of young animals. At the same time, good results may also be obtained with adult tissues.

Take pieces of *fresh* tissue, quite small [say a cube 4–8 mm.]. Place at once in the following mixture, in the dark:—

Pot. bichrom. 3 per cent. sol. . . . 4 parts.
Osmic acid, 1 „ „ „ . . . 1 part.

The proportions of these two constituents may vary

considerably. Use plenty of the solution (about 100 ccm.). The tissue remains in this mixture for two days; occasionally three or four, or even eight days. The time differs much according to the region of cortex to be stained, according to the age of the animal [for young embryoes, from 24–48 hours, for older animals, longer], and according to the elements which it is desired to stain—whether neuroglia, nerve-cell, or nerve-fibre. Taking these structures in the order given, the duration of stay in the mixture increases as one passes from the first to the last (v. Lenhossék).

The once-used solution should not be again employed. On removal from the mixture, wash the tissue for a few seconds in water, or in already used silver solution. Then place in *silver nitrate* solution. The strength of this varies from $\frac{2}{3}$–$\frac{3}{4}$ per cent. To it add formic acid in the proportion of one drop to 200 ccm. This prevents, in a measure, the formation of deposits of silver.* Use plenty of the silver solution. It need not be fresh each time. The solution with contained tissue is kept in an incubator at a temperature which varies with the elements it is desired to stain. If these are nerve-cells the temperature should be 25–28 degrees C., if neuroglia cells, about 35 degrees C. Suspend the tissue in the fluid by thread attached to the stopper of the bottle.

The silver solution is employed for 2–5 days, according to the thickness of the tissue. Longer than six days is said to be undesirable [? true for adult tissues]. Deposits of silver crystals are apt to form on the tissue and even in its interior, especially if penetration has

* Since writing the above, the author finds that v. Gehuchten, who recommended this practice, now discards it as superfluous.

been facilitated by an experimental injury to the cortex. These are mainly avoided by smearing the surface of the preparation with blood or celloidin solution before immersion in the silver. Sehrwald employes a 10 per cent. solution of gelatine in water; and various other plans are adopted.

As a result of the action of the silver the tissue becomes reddish-brown. Some recommend that a section should be made from time to time to see if the proper reaction (penetration) is being obtained. Hold the tissue whilst cutting between two pieces of hardened liver. If after several days in the silver solution reaction is still insufficient, the tissue may be put back in the hardening fluid 1–3 days, then returned to the silver.

On removal from the silver solution the tissue is washed in spirit for a few minutes, and the superficial deposit of silver is brushed off. If it is not of good cutting consistence, place in absolute alcohol $\frac{1}{4}$–$\frac{1}{2}$ hour. Sometimes celloidin infiltration may be needed, as when the tissue is from the cerebellum. If so, the procedure should be very rapid—5–15 minutes, at any rate, under one hour, in a solution of celloidin (alc. + ether) of medium thickness. Otherwise fine cell processes and the like delicate structures are injured. Thorough infiltration is the less needed, as with this method sections need not be as thin as is requisite with ordinary methods [0·05–0·1 mm. = approximate thickness]. Usually the piece of tissue can be cut without infiltration. In either case it is fixed to a cork with gum arabic or celloidin. Place the whole for a *short time* [no longer than necessary] in alcohol, 80 per cent., to harden the fixative.

Cut in a well, or sliding, microtome. Sections are

received into spirit. Pass them rapidly through absolute alcohol into a mixture of xylol and pure pyridin, equal parts. This mixture clears effectually and keeps sections elastic, whereas xylol alone produces brittleness (Andriezen).* As soon as the sections are clear, transfer to slide, remove excess of clearing agent by pressure with blotting paper, and cover with thick solution of dammar in xylol. Penetration of the dammar and evaporation of the xylol is hastened by placing the slide in the incubator at about 37 degrees C. *No cover-slip* is applied. Experience shows that specimens spoil even when the cover-slip is applied many days after application of the dammar. The procedure forbids examination under the highest power, but this becomes possible if the section is mounted on a cover-slip which is placed, preparation downwards, on two glass supports fixed to the slide. Keep the mounted specimens in the dark.

This important method requires much experience and minute attention to details. Successful preparations fully reward the pains expended on their preparation.

The parts acted on by the silver appear black or brown-black by transmitted light. These are: nerve-cells with their processes and ramifications, glia-cells and processes. These structures are probably made more evident by this method than by any other. Blood-vessels are also shown, with the attached processes of glia-cells.

The silver does not act by any means uniformly over

* For further recent observations upon the technique of Golgi's method (silver), *see* the same author, *Internat. Monatschr. f. Anat. u. Phys.*, 1893, Bd. x, H. ii.

a section, a statement particularly applicable to adult tissues. The elements mentioned are usually brought out only here and there. This isolation facilitates the examination of individual elements.

Specimens are commonly said not to keep. At any rate, they do not change for several months (over a year). v. Lenhossék states that he possesses some two years old which are unchanged in appearance.

Golgi's own methods, as given recently by Sala, are as follows :—After the detailed account of the Golgi-Cajal process, a full description of these methods is unnecessary ; the solutions employed in the preparation of the tissue differ somewhat in composition and method of use from those used in the latter process, but subsequent technic is much the same.

(*a.*) Slow procedure. Pieces of fresh brain not more than 1–2 ccm. in size are kept for 20–30 days in 2 per cent. sol. potass. bichrom., changed frequently. Or, after hardening in this solution 3–4 days, they are transferred to a stronger solution—3 per cent.—for four days. Increase the amount of the salt till 4–6 per cent is reached. 30–50 days may be spent in hardening. The tissues are then placed in aq. sol. silver nitrate, 0·75 per cent., for 24–48 hours.

(*b.*) Quicker (or mixed) procedure. The object is kept only 4–5 days in the bichromate, 2 per cent., solution and then transferred to following mixture :—Osmic acid 1 per cent. sol., 2 parts ; potass. bichrom., 2 per cent. sol., 8 parts. Here it lies 24–30 hours, and is then treated with the silver solution (0·75 per cent.) as before.

Some claim to have employed Weigert's hæmatoxylin stain successfully in combination with Golgi's silver methods. Sections are carefully washed in water after

the silver process, and then kept for twenty-four hours in chromic acid solution, ½ per cent. By this means the chrome dissolved out in the silver bath is replaced. They are then (without copper treatment) transferred to the hæmatoxylin, and treated as usual.

Bichloride of Mercury [Sublimate] (*Golgi's Method*). —Portions of brain from 1–2 cm. square, and about 0·5 cm. thick, are placed in a large quantity of bichromate of potassium solution, progressively raised in strength from 1–3 per cent. Müller's fluid may be used instead. Change the fluid frequently. Harden for 20–30 days. Pass the piece of tissue direct from the chrome solution to an aqueous solution of bichloride of mercury, 0·25–0·75 per cent. ; it is uncertain which strength is best. Here it remains at least 8–10 days, but the longer the stay in mercury the better the reaction obtained [several months not detrimental]. The bichromate diffuses out into the bichloride solution, which should be changed daily. Finally the tissue is quite decolourised.

Fix the piece on a cork or wood block, and cut sections. These must be thoroughly washed in water. Mount at once in glycerine, or dehydrate, clear, mount in balsam.

The ganglion-cells with their processes and branches, and the connective-tissue cells with their processes, are stained black. Blood-vessels are also shown. Other parts are unstained. The results are uncertain. Sometimes ganglion-cells, at other times glia-cells are stained, and then only here and there in the section. The best results are obtained with the cerebral cortex ; for cerebellum, the method is generally regarded as especially unsatisfactory.

Tal gives the following modification of the above, by

which a darker and sharper stain is obtained:—Sections are placed after washing in a solution of sodium-sulphide for a few minutes. This is prepared as follows:—100 grm. caustic soda are dissolved in 1,000 grm. water; one-half of this solution is saturated with sulphuretted hydrogen gas and added to the other half. Keep in a well-stoppered bottle.

It is claimed that the most satisfactory results are obtained when the sublimate method is conducted as follows:—The cerebral vessels of an animal just dead are washed out with bichromate of potash solution (2·5 per cent.). Small pieces of brain are then placed in Müller's fluid (often changed), for 8–10 days; then for twenty-four hours in a mixture of Müller's fluid 8 parts, osmic acid sol. 1 per cent. 2 parts. From this transfer to the sublimate solution. Preparations keep best without a cover-slip.

Sublimate sections may be double-stained with some suitable dye, or they may be stained after Weigert's hæmatoxylin process. Proceed as in the case of silver sections similarly treated.

Flechsig conducts the sublimate method in the following rather lengthy and complicated fashion:—Harden in 2 per cent. aq. sol. bichromate of potassium Impregnate with sublimate (same conditions as in Golgi's method). Cut sections, which are transferred to alcohol 96 per cent. Stain 3–8 days at 35 degrees C. in following solution:—

Pure extract of Japanese logwood .	1 grm.
Abs. alcohol	10 ccm.
Dist. water	900 ccm.
Sat. sol. sodium sulphate	5 grm.
„ „ tartaric acid	5 grm.

Each separate section is then placed in 3 ccm. of $\frac{1}{4}$ per cent. sol. of permanganate of potash, until the solution loses its blue tint. Decolourise in the following solution:—Oxalic acid 1 grm., potass. sulphite 1 grm., dist. water 200 ccm. Repeat the pot. permang., and the decolourising fluid until all yellow colour has left the section. Transfer to following mixture:—

Chloride of gold and potassium, 1 per cent. sol.	5 drops.
Abs. alcohol	20 ccm.

In which the section remains until the sublimate deposits, which appear white by reflected light, have become deep black, and the red-stained nerve-fibre bundles have assumed a bluish tint. Wash rapidly in

Cyanide of potassium, 5 per cent. sol.	1 drop.
Aq. destill.	20 ccm.

The section must float on the surface of the solution. Dehydrate in abs. alc., clear in lavender oil, mount in balsam.

Ganglion-cells and their processes are deep black, nerve-fibres carmine-red.

Obregia treats sections prepared by Golgi's method [whether sublimate or silver] as follows:—The stain obtained is from dark green to dark violet. Pass sections from absolute alcohol to the following solution of gold chloride:—

Chloride of gold 1 per cent. sol.	8–10 drops.
Abs. alcohol	10 ccm.

This should have been made half-an-hour previously, and exposed to diffuse light for that time. Place

sections in this and remove to the dark. The silver is gradually replaced by gold, the mercury changed to a gold-amalgam. The fluid is allowed to act 15–30 minutes, according to the thickness of the section; longer does not hurt much. Wash rapidly in alcohol 50 per cent., then in distilled water, and in 10 per cent. sol. hyposulphite of soda. In the last sections remain 5–10 minutes, according to their thickness. If it is allowed to act longer the staining is too faint, and thus fine fibres are lost. Finally, thorough washing in water, twice renewed. Sections may now be stained in any way—as by carmine, hæmatoxylin, Weigert's hæmatoxylin. Dehydrate in alcohol, clear in creosote, mount in dammar. A cover-slip may be used.

Ziehen's Method. — Ziehen, referring to Golgi's silver method, says that it suffers from two disadvantages, apart from non-durability. In the first place, the black staining of the cells covers all details of their internal structure; in the second place, medullated fibres are unstained [Flechsig, however, has combined Weigert's hæmatoxylin method with the silver method with good results.] Ziehen proposes to overcome these defects by the following process:— Small cubes of *fresh* brain-tissue are placed direct in the following solution and there hardened (no chrome used throughout):—

Gold chloride 1 per cent. sol. . . . . } āā.
Sublimate 1 per cent. sol. . . . . }

Three weeks at least needed; some months—up to five—better still. A gold-amalgam is formed in the elements. Change occasionally. Preparations become a metallic red-brown. Mount, without imbedding, on

cork. Sections are cut into alcohol. Transfer to lugol solution [*see* "Hardening Methods"; sublimate + alcohol process], diluted with water, 1 : 4. Or use Tr. Iodi, diluted with alcohol to the same degree. Here they remain a varying time according to their thickness. This is an important stage and demands experience. By controlling the action of the iodine staining can be practically restricted to the cell-contour and to the nucleus and nucleolus—as far as the cell-elements are concerned. The cell-body, in any case, is almost translucent, and commonly of a faint blue-black tint. Wash in absolute alcohol. Clear in clove oil; balsam.

In addition to nerve-cells, nerve-fibres—medullated and non-medullated—and glia-cells and their processes are stained, of a bluish-grey. More nerve-cells are in general seen than with Golgi's silver method. Metal instruments should be avoided. The contact with the knife used in cutting does not seem harmful.

Gold.

Freud's Method.—Sections of material hardened in Erlitzky's fluid (which may be followed by alcohol, if desired), are washed in water and put for 3–5 hours into aq. solution of chloride of gold 1 per cent. Wash again in water and place for three minutes in a solution of caustic soda 1 part, water 5–6 parts. After draining off excess of this fluid they are placed in a solution of iodide of potassium, 10–12 per cent., where they remain 5–15 minutes. Here they assume a reddish-violet tint. Wash in water, dehydrate [use alcohols of increasing strength, to absolute], clear, and mount in balsam. If objects stain easily, dilute the

gold solution with one volume of alcohol; a more selective stain is obtained. Glass or wooden needles should be used in the manipulations.

The results are very variable. When successful, the preparations are instructive. One sees the bundles of nerve-fibres radiating into the cortex from the medullary centre, and in the cortex itself, under a high power, a meshwork of fine fibrils of reddish hue, sometimes dark blue or black. This can be traced far up the cortex, in an outward direction. Nerve-cells are often unstained, at other times sharply stained, of a reddish colour. The reason of these variations is unknown.* The ground substance is but slightly coloured in a good specimen.

Sections keep well for many months, at any rate.

Gerlach's Method.—Harden quite small and fresh pieces in an aq. sol. ammon. bichromate. Boll advises that less than 1 per cent. be used at first. In a few hours raise the strength to 1 per cent., and in a couple of days to 2 per cent. Harden no longer than necessary, as the gold reaction does not succeed well after about the eighth day [Boll]. As soon as the tissue is firm enough it may be cut into still smaller pieces, and hardening thus hastened. Sections are cut by hand (in the original method); as the tissue should not come into contact with alcohol the piece may be cut between two pieces of liver; these may be held in the clamp of a sliding microtome. Wet the knife with distilled water, and cut the sections into the same. The thinnest are placed in a solution of double

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* According to Freud, nerve-cells stain in the adult; in the new-born and in embryoes, only nerve-fibres.

chloride of gold and potassium, 1 : 10,000, very slightly acidulated with HCl. Here they remain twelve hours. Use a small quantity of gold solution relatively to the tissue [Boll]. Next wash sections in HCl. sol., 2 : 3,000, after which they are placed for ten minutes in a mixture of 1 part HCl. and 1,000 parts alcohol 60 per cent. Absolute alcohol, clove oil, balsam, as usual.

The writer has had no success with this method as applied to the absolutely fresh cortex of kittens. It may be designated most uncertain. With human brain as ordinarily obtained it is futile to expect good results. Boll appears to have obtained good specimens with the fresh brains of small mammals [mouse, rabbit]. From the statements of this writer it seems that Gerlach himself obtained successful preparations from the human cortex.

The method is said to demonstrate the nerve-fibre bundles radiating from the white to the grey matter, also networks of fine nerve-fibres in the cortex, medullated and (still finer) non-medullated—the latter formed by the prolongations of ultimate branches of nerve-cells.

Possibly success is more easily obtained with embryonic brain. The method is much more commonly employed with spinal cord than cerebral cortex.

In an article upon gold-staining, *Journal of Nervous and Mental Disease, Oct.*, 1890, Upson observes that the stain "is dependent upon the presence of extraneous matters in the gold solution. Pure gold chloride dissolved in distilled water and carefully neutralised stains little if at all. It is not too much to say that every section which has been successfully stained by the older

methods has been so by virtue of hydrochloric acid or some other impurity accidentally present in the gold solution. Commercial chloride of gold almost always contains hydrochloric acid, often also chloride of copper . . . . ; much better results may be obtained by adding substances designedly to the gold solution, and these substances should be in general either acids, or metallic salts, or both." Upson gives the two following methods of employing gold as a stain for nerve-cells and axis-cylinders. Very fine results are obtainable when they are properly carried out, but there can be no doubt that more skill is required than is requisite for ordinary methods.

Upson's Gold Methods.—The hardening process is alike in both methods. Small pieces are placed in potass. bichrom. 2 per cent. sol. (dist. water). For the first few days it is advisable to use a 1 per cent. sol. Change often, and after some weeks increase to 2·5 per cent. Hardening is carried out in the dark ; it must not be overdone, as over-hardened tissue is of no use for these methods. Wash rapidly, when hardened, in dist. water. Transfer to alcohol 50 per cent. solution for 2–3 days, changing occasionally. Then to alcohol 95 per cent. solution. Here pieces remain until they acquire a greenish colour (2–4 weeks). Change alcohol often. The green, hard pieces are cut—without imbedding or in celloidin—into 80 per cent. alcohol. Here the sections may remain some days; or they may be stained at once. The latter is the better plan. The staining methods are as follows :—

(i.) Place sections in gold chloride 1 per cent. solution, to which is added HCl in the proportion of 2 per cent. Here they remain 1–2 hours; they acquire a yellow tint.

Wash lightly in distilled water, and place in potassium hydrate solution 10 per cent. [10 ccm., to which add a trace of ferricyanide of potassium—a piece about the size of a pin's head, powdered.] This is freshly made before use. Sections remain here ½–1 minute. Wash well. Transfer to 10 per cent. solution potassium hydrate for half a minute. Wash again for a moment, and place in the following reducing fluid, made fresh just before use.

Sulphurous acid	5 ccm.
Tr. Iodi. 3 per cent.	10–15 drops

mix and add liq. ferri. chloride 1 drop.

Place at once in this, after washing. Here the section quickly gets a fine rose colour. Remove then at once to dist. water. Wash; abs. alcohol 5–10 minutes, clove oil, balsam.

The addition of the ferricyanide of potassium to the potash solution is not essential.

(ii.) Prepare following:—Sol. *a.*: To a certain quantity of Tr. Iodine 3 per cent. add so much protochloride of tin that the colour becomes white or yellow. Sol. *b.*: Sat. sol. phosphate of iron in dist. water.

The section is placed in following stain for two hours:

HCl.	2–3 drops.
Chloride of gold 1 per cent. solution	5 ccm.
Sat. sol. ammonium vanadate . .	10 drops.

Wash in dist. water for a moment. Place in following solution (fresh before use):—

Potassium hydrate 10 per cent. solution	5 ccm.
Ammonium vanadate	a trace.
Permanganate of potash 10 per cent. solution	10 drops

In this the section remains ½–1 minute. Wash momentarily in dist. water, then place in the following reducing fluid, freshly made before use :—

Tin solution, *a*	15 drops.
Aq. destill..	3 ccm.
Iron solution, *b*	3–5 drops.
Sulphurous acid	3 ccm.

On adding the acid a thick deposit occurs. At this moment the reducing fluid is strongest, and the section should just then be put in. It quickly assumes a purple colour. When this appears remove at once to distilled water, and proceed as in method (i.), [*i.e.*, alc., oil, &c.]. A spatula of platinum or glass should be used.

Iodide of Palladium (*Paladino's Method*).—Harden pieces not more than 5–8 mm. thick in Müller's fluid, or in pot. bichrom. 2–4 per cent. sol. Wash out excess of chrome in running water. Dehydrate in repeated baths of alcohol, absolute or 96 per cent Then dissolve away all the soluble portion of the myelin as follows :—Boil small pieces successively in a mixture of abs. alcohol and benzole, in benzole, and in alcohol—absolute or 96 per cent. The pieces lie for one hour in each of these solutions. They are then placed in a relatively large quantity of aq. sol. chloride of palladium, obtained by addition to the water of hydrochloric acid in drops, and agitation in a mortar, until the palladium is quite dissolved. Strength—palladium, 1–2 parts ; water, 1,000.

Pieces remain in this solution till no further decolourisation occurs (3–4 days). Protracted immersion, even beyond a week, is advantageous for the complete impregnation of pieces, and the solution may be

renewed occasionally. When properly impregnated, transfer to a solution of potassium iodide, 4 per cent. The quantity of liquid used is important, also the time for which it is used, because the palladium iodide formed is soluble in excess of potassium iodide. Use a small quantity of fluid only, and do not change it. The length of stay in this solution is from 1–2 days or more—experience must decide. (It is as well first to see the effect of a shorter stay—2–3 hours.)

The piece of tissue is now dipped successively in alcohol of 80 per cent. and 96 per cent. for dehydration, and imbedded. Celloidin is better than paraffin for this purpose, as the latter spoils the iodide of palladium reaction in the superficial parts of the tissue. Place in celloidin dissolved in ether for a few days, after dipping preliminarily in ether. Mount on block and harden celloidin in alcohol, 50 per cent. Sections are then cut. Dehydrate and clear as usual, and mount in balsam dissolved in chloroform.

A brown-black or brown-yellow stain results.

By this method various details of the structure and relationship of the neuroglia-cells, the structure of the myelin sheath and of the axis-cylinder are brought out. Paladino has been able to show a relationship between the neuroglia and the skeleton of the myelin sheath; that the latter is a continuation of the former and contains also glia-cells. The neuroglia, in fact, according to Paladino, constitutes the framework of the myelin, both in the white and grey matter. The method also shows the meshwork formed by the neuroglia around the nerve-cells, the fibrils of which terminate on the surface of the cell. Interstitial and nervous elements proper are shown contemporaneously.

The method appears hitherto to have been chiefly employed on the spinal cord.

Vassale* and Colella† have employed Paladino's process with good results. At present it appears but little known in this country.

Copper Sulphate (*Monti's Method*).—Pieces of moderate size are placed in Müller, or pot. bichrom. sol., of ordinary strength, and allowed to harden slowly. The tissue must not have gone into the green stage. After hardening place in following mixture :—

Müller, or very concentrated sol. pot. bichrom . . . . . } āā.
Cupric sulphate, 20 per cent. sol. . . . }

Reaction begins in twenty-four hours and goes on during some days. The length of stay must be decided by experience. The piece on removal is cut, and sections are put up in balsam, dammar, or glycerine.

The elements in which reaction has occurred are yellowish-brown or blackish by transmitted, reddish by reflected light. As in the mercuric and silver methods, the different varieties of tissue-element are not stained

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* Nuovi metodi d'indagine microscopica per lo studio di alcune particolarità di struttura dei centri nervosi. Reggio d'Emilia, 1891.

† Ricerche sperimentali e istologiche sulla degenerazione e sulla rigenerazione dei ganglii del simpatico. Napoli, 1891.

The following references to Paladino's work may be of service :— Dei Limiti Precisi tra il nevroglio e gli elementi nervosi del midollo spinale : *Boll. della R. Accademia Medica di Roma*, Anno xix, fasc. ii. *Archiv. italiennes de Biologie*, t. xiii, p. 484. Di un nuovo processo per le indagine microscopiche del sistema nervoso centrale. *Rend. d. R. Accad. delle scienze fisiche e matematiche, Napoli*, vol. iv., p. 14, 1890. Della continuazione del nevroglio nello scheletro mielinico delle fibre nervose : *Ibid.*, fasc. 7–12, July–December 1892. Especially the first mentioned.

at one and the same time. After periods of different duration ganglion-cells, nerve-fibres, and glia-cells respectively are stained. The method is recommended especially for neuroglia-cells and nerve-fibres and for the control of Golgi specimens. Further experience of it is needed.

Chloride of Zinc. — *Magini* has recommended chloride of zinc for the histological study of the brain, and gives the following method, which has been as yet but little tested. He states that it is adapted for the study of nerve-cells, including their nuclei and nucleoli :—

Pieces 2–3 ccm. in size are hardened in Müller for at least 2–3 months. Wash in distilled water, then place in sol. of zinc chloride, 0·5–1 per cent. Here they lie 7–10 days. Renew the fluid every day. Sections are cut, washed quickly in alcohol, half-cleared in creosote [*see* " Half-Clearing Methods "], and mounted in dammar.

Stains Used According to Special Methods, and Other (Miscellaneous) Stains.

Nissl's Method of Staining Nerve-Cells.—Portions of tissue as fresh as possible and 1–2 ccm. in size are placed, some in absolute alcohol, and some in nitric acid, 10 per cent. solution. For subsequent treatment of the latter pieces *see* " Fixation Methods " (nitric acid). The former are removed from the alcohol after twenty-four hours, fixed on cork by gum [*see* " Section Cutting "], and sections cut. These are passed from alcohol into aq. sol. methylene-blue. [" Methylenblau patent B "] 0·5 per cent. The stain with contained sections is heated in a watch-glass until the crackle of

bursting bubbles is heard, not longer. After cooling, sections are transferred to a mixture of anilin-oil and alcohol 96 per cent. (20 : 200). This should not be too old. Here they are agitated until no more clouds of colour are given off. In this mixture differentiation is brought about. Remove section to slide and dry well with filter paper (by pressure). Allow a few drops of origanum oil to flow over it momentarily, drain this off, and dry a second time with filter paper. Remove final traces of origanum by means of benzine, and then allow to fall over the section a drop of colophonium solution (colophonium dissolved in benzine; the consistence of ordinary balsam). The slide is now drawn through the flame of a spirit-lamp; the benzine is set on fire. Allow it to burn off.* A cover-glass is now placed over the preparation, and the slide carefully warmed until the colophonium is fluid and the cover-glass easily moveable in it. Allow to cool.

By this method the structure of the nerve-cell *body* is well shown. The staining is blue. For cell-*nuclei* special fixative-fluids, as Flemming's mixture, sublimate, and especially—according to Nissl—nitric acid should be used. He states that if the latter fixative is employed staining of the cell-body (by methylene-blue) can be secured at the same time that the nucleus is shown by some hæmatoxylin stain—a result not obtainable after fixation in other reagents.

Connective-tissue nuclei and those of vessel-walls are also shown well by the method.

Rehm recommends that the methylene-blue solution

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* It appears, practically, quite sufficient to produce evaporation of the benzine by heating the under surface of the slide.

(0·1 per cent.) be first heated and the section then placed in it. This plan has the advantage that the section does not go to the bottom and roll up; further, it permits to some extent, at any rate, differentiation between nerve and connective-tissue cells, as the former take up the warm stain much quicker than the latter. By leaving the section therefore in the warm stain a short time only ($\frac{1}{2}$–1 min.), a deep staining of nerve-cells, as contrasted with faint staining of connective-tissue cells is obtained. This differentiation is accentuated by using 96 per cent. alcohol simply, instead of anilin-alcohol, after the stain. Nerve-cells then appear dark blue, connective-tissue cells greenish. These colour-differences are better appreciated by the yellow light of lamp or gas.

Nissl's method affords far more information concerning the structure of the nerve-cell than is obtainable by the ordinary methods (chrome-hardening, staining by carmine or anilin blue-black). The granulations of the cell-protoplasm are probably shown better by this than by any other method. As pathological changes often affect these fine structures quite early in a disease the method permits the recognition of diseased cells earlier than is possible with other methods. It should, therefore, be used in such cases as those dying in acute mania, in which it is very often difficult, in fact, impossible, to recognise pathological changes in the nerve-cells. Moreover, morbid states of the nuclei of the ganglion-cells are indicated by the methylene-blue process; whereas normal nuclei are unstained, diseased ones are deeply stained.

The method is not adapted for showing fatty and pigmentary changes in the cell, owing to the preliminary treatment by alcohol.

Formerly Nissl stained with magenta instead of methylene-blue, and this method also gives useful results. It is as follows :—

Harden pieces in alcohol, as before, cut with or without imbedding in celloidin. If the latter is used infiltration must be very short. Fine sections are required. Place them in sat. aq. sol. magenta-red, and warm the solution until a slight steam arises. Wash in absolute alcohol. Differentiate in clove-oil till no more stain is given off.

Nerve-cells with their processes are very distinct The structure of cell-body and nucleolus is brought out, the nucleus is not well shown. Vessels are well stained. Ground substance almost completely decolourised.

Rehm's Method of differentiating Nerve from Connective-Tissue Cells.—Small pieces of tissue are hardened in alcohol (96 per cent. followed by absolute) and imbedded in wax or celloidin, or fixed to a cork, and sections cut into alcohol. These are placed in warm 0·1 per cent. aq. sol. methylene-blue for not more than half-a-minute. Decolourise in alcohol 96 per cent. Decolourisation is sufficient when nerve-cells appear a well marked blue, connective-tissue cells quite pale. [This point must be decided in the earlier attempts by microscopical examination.] Transfer to following solution: fuchsin 0·1 grm., alcohol 96 per cent. 100 ccm. Here sections remain $\frac{1}{4}$–$\frac{1}{2}$ hour. Wash in alcohol until no more red clouds are given off (one minute). Place momentarily in clove-oil, transfer to slide, dry well with blotting paper (by pressure), mount in chloroform-colophonium or canada-balsam.

In a successful preparation nerve-cells are stained blue — perhaps blue-red — all connective-tissue and

vascular nuclei deep red. The nuclei of nerve cells are unstained in a healthy specimen, though the nucleolus may be blue. In pathological states red granules are often seen in abundance in the nucleus of the ganglion-cells. It is important that the staining in methylene blue should be momentary as compared with that in fuchsin.

A specimen not quite successful may be corrected as follows:— Clove oil abstracts fuchsin, origanum oil, methylene blue. If a section is too blue place momentarily in origanum; if too red, in clove oil.

The same author recommends the two following methods for the isolated demonstration of connective-tissue cells and those of blood-vessels:—

(1.) Sections, prepared as before, are placed for some minutes in cold aq. sol. eosin, 1 per cent., washed in water, then in alcohol, and transferred to warm aq. sol dahlia, 0·1 per cent., where they remain some minutes. Differentiate in alcohol; origanum oil, balsam or colophonium.

Nuclei of connective-tissue and vessels dark blue, other structures red.

(2.) Instead of eosin use nigrosin (aq. sol. 1 per cent.), and instead of dahlia, fuchsin (0·1 per cent. alcoholic solution). In the latter sections remain half-an-hour. Differentiate in alcohol; clove oil, chloroform-colophonium.

Connective-tissue nuclei now appear red, other structures blue-grey.

Wolters's Methods for Cerebrum and Cerebellum.

(*a.*) **For Cerebellum.** — Harden small pieces in Müller's fluid, remove excess of chrome by water, after-

harden in alcohol. Cut sections in celloidin and place them in following fluid :—

Vanadium chlorate 10 per cent. . . 2 parts.
Aluminium acetate 8 per cent. . . 8 parts.

This mordant having acted twenty-four hours, wash in water (5–10 minutes), and place in Kultschitzky's hæmatoxylin for twenty-four hours at 45 degrees C.

Hæmatoxylin (Grübler) . . . . . 2 grm.
Alcohol abs. qs. ad. solv.
Acetic acid 2 per cent. sol. . . . 100 ccm.

It is then recommended to dip the sections in Müller's fluid and differentiate them according to Pal's process. Wash in water, dehydrate; xylol, xylol-balsam.

Purkinje's cells, with their protoplasmic processes are well stained. Myelin sheaths of nerve fibres also stained.

(*b.*) **For Cerebrum and Cerebellum.**—Place small pieces in Kultschitzky's hardening fluid [for composition, *see* his method of staining neuroglia] for 12–24 hours, in the dark. Transfer to strong alcohol for 12–24 hours. Cut sections in celloidin and place them in the above-mentioned mordant for twenty-four hours. Wash in water ten minutes. Stain in the above hæmatoxylin solution as before. Transfer sections to 80 per cent. acid-alcohol (alcohol 80 per cent. 200 parts, HCl. fort. 1 part), and decolourise till a clear bluish-red tone is obtained. [The necessary time must be found by experience.] Remove acid by weak alcohol; dehydrate (abs. alc.); origanum oil, balsam.

The pyramidal nerve-cells of cerebrum are stained of a deep blue-black; their branching protoplasmic processes may be followed a considerable distance

upwards. The lateral processes, and meshwork formed by them, are also well marked. The axis-cylinder process can be traced to the white matter. Other nerve-cells—with their processes—are also shown. Only when decolourisation is insufficient is there any colour left in the medullated nerves. Glia-cells also stained.

In the cerebellum are stained: the ganglion-cells with their protoplasmic and axis-cylinder processes, the latter traceable to the central medulla.

The method is comparable to Golgi's silver process but is more certain, according to Wolter's. A larger number of elements also appear stained in a single section.

Sahli's Method (for nerve-cells and nerve-fibres).—Sections of tissue hardened in potass. bichromate solution to the degree required for Weigert's hæmatoxylin method (*i.e.*, the tissue must have acquired a brown colouration) are washed in water a few minutes and then stained for several hours (6–12) in the following solution:—

Sat. aq. sol. methylene-blue . . .	24 ccm.
Borax 5 per cent. sol.	16 ccm.
Water	40 ccm.

Wash in water and then in alcohol until the grey matter is differentiated from the white, clear in cedar-oil, mount in balsam.

The nerve-tubes radiating outwards into the cortex show well for a considerable distance, they are stained deep blue; ganglion-cells are greenish-blue; connective-tissue nuclei deep blue; ground substance light blue.

The outermost layer may easily be too much de-

colourised if care is not exercised. In fact, the stain is removed by alcohol as easily as most anilin dyes, and therefore the preparation should be removed to cedar-oil as soon as permissable.

Kronthal's Method (for showing ganglion-cells).—Remove with the point of a knife from the motor cortex (upper end of ascending frontal gyrus) a piece about the size of a pin's head, place it on a slide, and press out beneath a cover-slip to a thin film. A drop of aq. sol. methylene-blue, 0·5 per cent., is placed at the edge of the cover, and the latter gently raised, so that the film is bathed by the penetrating stain. After about one minute remove excess of stain by blotting paper, and detach the cover from the slide in such a manner that most of the preparation remains on the cover. Allow the film—what remains on the slide may also be employed—to dry in the air at the ordinary temperature. When dry, cover with balsam.

A better plan is to rub the excised material between two cover-slips; these are then separated, and the film allowed to dry sufficiently to procure adhesion to the glass. Cover with the stain. In abont one minute wash off excess, dry, and mount in balsam.

Ganglion-cells with their nuclei and processes, and connective-tissue nuclei and blood-capillaries are shown.

Alizarin has been recommended by Benczur as a stain for the central nervous system. Stain chrome-hardened sections for twenty-four hours or more in concentr. sol. of alizarin in alcohol. (A slow stain.) Sections then appear of a brick-dust or reddish-brown colour. The stain does not dissolve out much in water or alcohol.

Nerve-cells appear reddish-brown; their nuclei, connective-tissue nuclei, and axis-cylinders the same, but a

darker shade; ground substance a bright brown. The interlacing nerve-fibres at the lower part of the cortex also reddish-brown. Staining is rather faint.

The writer does not find that alizarin presents any advantage over the commoner dyes.

Purpurin has been recommended by Duval as having a special action on nerve-tissue, particularly spinal cord. Whilst the nuclei of connective-tissue and capillaries stain red, nerve-cells, their processes, and axis-cylinders remain unstained. The writer hardened portions of the fresh cortex of a cat in bichromate of ammonia (2–1,000) as recommended by Duval, and stained with purpurin. The selective staining referred to was not obtained, although connective-tissue nuclei came out well.

The purpurin solution is as follows (Ranvier, quoted by Bolles Lee):—Alum grm. i., water 200 ccm. Boil. Add the purpurin rubbed up in some water, and boil further. See that the purpurin is in excess. A saturated solution of purpurin is obtained. Filter whilst hot into 60 ccm. of alcohol 90 per cent. The solution keeps only a few weeks. Stain sections forty-eight hours. Treat subsequently as usual.

Picro-Nigrosin is used by Martinotti as a stain for the central nervous system; it is said to give especially good results with pathological tissue. Stain for 2–3 days in sat. sol. nigrosin in sat. sol. picric acid in alcohol. Wash out in a mixture of 1 part formic acid and 2 parts alcohol, until grey and white matter are clearly differentiated. Dehydrate, clear, mount.

The writer does not find that this method presents any advantage.

The two following staining methods are designed

especially to show axis-cylinders. They may be given here, though more applicable to the case of the spinal cord and peripheral nerves than of the brain.

Stroebe's Axis-Cylinder Stain.—Harder in Müller, thereafter in alcohol, if desired, and cut sections as usual. Stain in fresh sat. aq. sol. anilin blue for ten minutes to one hour. Sections become blue-black. Wash off excess of stain in water, then place in a small porcelain dish of absolute alcohol, to which have been added 20–30 drops of 1 per cent. solution of alkali-alcohol (1 grm. caustic potash to 100 ccm. alcohol). In the alkali-alcohol sections turn of a light rusty colour, clouds of reddish colouring matter issuing from them. As soon as these cease to form and the sections are of a light red-brown colour, and transparent, differentiation is complete (one to several minutes). Wash in distilled water (five minutes); sections acquire a clear blue tint. Contrast-staining is then performed in conc. aq. sol. of safranin, diluted with an equal part of water, $\frac{1}{4}$–$\frac{1}{2}$ hour. Place in absolute alcohol to remove excess of safranin and to dehydrate; sections now look red, with a tinge of blue; xylol, xylol-balsam.

Axis-cylinders appear dark blue; medullary sheaths, cell protoplasm, ground-substance, and cell-nuclei, various shades of red; the last-named sometimes retain the blue colour.

The results are not to be obtained with certainty.

van Gieson's Stain.—Harden in Müller, as in last case, and cut sections as usual. Stain them 3–5 minutes in a hæmatoxylin solution (Delafield's, or other alum-hæmatoxylin). Wash well. Stain in a mixture of sat. aq. sol. picric acid, and sat. aq. sol. acid-fuchsin, sufficient of latter to make a deep red fluid. Wash

rapidly in water. Spirit, alcohol, origanum oil, canada-balsam.

Axis-cylinders appear deep red, medullated sheaths yellow; the glia is of a reddish tint, nuclei are blue-violet; any sclerosed tissue is intense red. Amyloid material is stained a light red.

Kultschitzky's Stain for Neuroglia.—Rubin is the stain employed in this method [" Patent saures Rubin " from the Berliner Anilinfarben - Actiengesellschaft. Grübler will supply]. Portions of brain are fixed in the following [Kultschitzky's hardening mixture]:— A saturated solution of bichromate of potash and sulphate of copper in 50 per cent. alcohol, to which is added at the time of use glacial acetic acid, 5–6 drops to 100 ccm. The solution is carried out in the dark, about twenty-four hours required. The time employed in fixation depends upon the size of the object; twenty-four hours suffices for small objects, but for large ones 2–3 months may be taken. Fixation is carried out in the dark. Transfer the object to strong alcohol, without preliminary washing; here it hardens. Darkness is unnecessary during this stage. When sufficiently hard, pass through paraffin, as usual. Cut sections. These, freed from paraffin, are placed in the stain, composed as follows :—

Acetic acid sol., 2 per cent. . .	100 ccm.
Patent saures Rubin.	0·25 grm.
Sat. aq. sol. picric acid	100 ccm.

The picric acid remains in the nerve elements, and so affords some contrast to the neuroglia. The stain acts very energetically, usually a few seconds in it suffices. Wash in 96 per cent. alcohol, changed once; the rubin

is almost insoluble in alcohol, and so is not extracted. Remove all excess, and transfer to absolute alcohol. Clear, mount in balsam.

The cells and fibres of the neuroglia are stained reddish-violet. Other elements are but slightly stained. If, however, the dye is used for more than a very brief time nerve-cells and axis-cylinders become stained, of a yellowish-red.

Sections must be very thin.

More recently K. has used the following stain :—

Alcohol, 96 per cent.	100 parts.
The above-mentioned rubin sol. .	3–5 ccm.

With this the staining process is much lengthened (half hour or more), but sections are less apt to break up.

Beneke's Method of Staining Neuroglia.—This is based upon Weigert's fibrin-method. In that method a mixture consisting of anilin oil, 2 parts, xylol, 1 part, is used as a decolourising agent. The former is the acting constituent, the xylol merely controlling the decolourising action of the anilin oil. It has been observed that various other tissue elements, as well as fibrin, are stained by Weigert's method; amongst these, Beneke noted connective-tissue fibres. By varying the proportions of xylol and anilin oil, various tissue elements may be stained, which are not shown by the original method. Beneke finds that, by systematic reduction of the strength of the decolourising fluid (by increasing the proportion of xylol to anilin), connective-tissue in various organs—including the brain—can be consistently stained.

To stain the glia-cells and fibres, proceed as follows:—Small pieces of tissue, which have been fixed in alcohol,

are passed through paraffin as usual; sections are cut, and fixed on the slide [by simple melting of paraffin; no adhesive material used]. Remove the paraffin, as usual, and stain with anilin water gentian-violet, 10–20 minutes (10 parts anilin oil, shaken into a fine emulsion, with 100 parts water; filter. To filtrate, add 5–10 drops conc. alcoholic sol. gentian-violet).

Pour off excess of stain, and treat for about one minute with lugol, diluted to a port-wine tint. (Lugol: iodine, 4 parts; KI, 6 parts; water, 100 parts.) Dry carefully with blotting paper (by pressure), and pour on anilin-xylol (anilin oil, 2 parts; xylol, 3 parts). The violet colour comes away in streams. *As soon as* the section is dehydrated and cleared, wash off the anilin-xylol by pure xylol. This is the most important stage of the process. To avoid undue decolourisation it is as well to pour xylol over the preparation occasionally, whilst under the action of the anilin-xylol, to ascertain whether it is dehydrated and sufficiently decolourised. Mount in xylol-balsam. Preparations are said to be permanent.

The glia-cells and processes, and the fine fibrous meshworks found at various parts—as, for example, between pia and cortex, and around the ventricles—are stained blue-violet, occasionally reddish-violet. Sclerosed areæ are well shown. The nerve elements proper, with exception of the nuclei of the nerve-cells, are unstained.*

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* For a "Special Method" of staining the nervous system recently introduced, *see* "Addendum," at the close of this work.

VII.—HARDENING COMBINED WITH STAINING.

The object of this dual procedure is not merely the saving of time, though at first sight it is not obvious what other advantage it possesses over the ordinary methods. In fact, the addition of the stain to the hardening fluid seems to have been, in the first instance, an empirical proceeding. It was hoped that in this way a better staining of structures shown by the usual process of staining after hardening might be obtained; also that some tissue elements not shown, or indifferently shown, by that process might be demonstrated. In the writer's experience, structures ordinarily seen are no better shown by the dual procedure in question, though they come out quite as well if proper care is taken. This being so, the method commends itself on the simple score of time. The writer has been unable to make out by this method any further details of structure. Some, however, state that the perivascular lymph-sheaths and the pericellular lymph-sacs may be seen considerably better in sections from tissues hardened and stained simultaneously, than in those stained separately after hardening. This result, to be more precise, has been obtained with the cortex of the cat.

Although the writer and others working in the

laboratory at this asylum have failed to get good results with the method in question up to the present (as far, that is, as the point last mentioned is concerned), it certainly appears worthy of further trial.

No reliable procedure by which combined staining and hardening can be obtained with, at any rate, some degree of uniformity has yet been formulated. The object, of course, is to have the tissue stained throughout by the time that it is hard enough for cutting. Usually one finds that the one process is complete before the other. Nevertheless, a tissue which is hard enough, though not stained uniformly throughout, may repay examination. One of the stained areæ may show the perivascular and pericellular channels and saccules which it is desired to demonstrate.

The following observations may be of service in further trial of this method.

Erlitzky's fluid is not likely to recommend itself as the hardening reagent. It hardens so quickly that even a strong staining fluid cannot penetrate the tissue in time. For this the rapid induration of the outermost layers of the cortex is, perhaps, especially responsible. It is best to use Müller's fluid or a solution of a chrome-salt (as pot. bichrom., 4 per cent.). These may be preceded by methylated spirit, and the stain added to the spirit as well as to the chrome solution.

Pieces to be treated should be small—1–1½ cm. square, and about 4 mm. thick. Examine a piece under treatment from time to time, at any rate in the earlier trials. Cut a thin slice off one of the sides to see if the stain is penetrating. If hardening seems in advance of staining, use a solution in which the percentage of stain is higher. If the reverse is the case,

increase the percentage of hardening reagent. As regards the stain to be used : hæmatoxylin alone appears quite unsatisfactory ; it penetrates badly when employed with chrome solutions. (Concerning the results when alcohol alone is used in hardening the writer has no reliable experience with this or other stain.) Cochineal and carmine are precipitated in these solutions. Anilin blue stains too diffusely, and the same holds good for the anilin dyes as a class. Precise staining, in their case, can only be obtained by subsequent decolourisation (differentiation), either of the mass or of sections, a procedure difficult to control, especially in the first case.

Anilin blue-black appears to be the most suitable stain to combine with a chrome solution. After much experimentation, the writer obtained fairly good results as regards general staining, as follows :—Pieces of fresh brain of the dimensions given, were placed in absolute alcohol 4–5 hours, and then transferred to the following solution used freely :—anilin blue-black $\frac{1}{4}$ gram., pot. bichrom. sol., 4 per cent., 100 ccm. In five days, as not much more than the periphery appeared stained, the pieces were transferred to a stronger solution of anilin ; anilin blue-black, 1 gram. ; pot. bichrom., 4 per cent. sol., 100 ccm. To this chromic acid, 1 per cent sol. was added (15 ccm. : 80 ccm. of original sol.), as it was thought possible the tissue might be stained before it was hardened. The whole was placed at 35 degrees C. In seven days more the piece was ready for cutting, and the stain appeared to have gone through it. On examination, however, the staining was found to be irregular ; here and there the nerve-cells and their nuclei were well stained, elsewhere but

faintly. Connective-tissue nuclei and blood-vessels (including perivascular sheaths) were no better brought out than by the ordinary process, nor were the pericellular sacs well shown. The tint was a very agreeable, soft blue-black, rather darker than that seen when separate, previously hardened sections are stained.

The above results were obtained with the cornu ammonis and motor cortex of the cat. Equally good results were got by the following method (human motor cortex):—Pieces, 1½ cm. square by 5 mm. thick, placed in following solution twenty-four hours:—methylated spirit, 2 parts; anilin blue-black, ¼ per cent. sol., 1 part. They were then transferred to a mixture of pot. bichrom. 4 per cent. sol., 1 part, and anilin blue-black, ¼ per cent. sol., 2 parts. In one month hardening and staining appeared complete, and sections were cut. Although, however, the stain had acted well on the cortex it had not penetrated thoroughly into the medulla.

A combination of anilin blue-black and hæmatoxylin may very possibly be found to penetrate more rapidly and give better results than the simple anilin.

VIII.—CLEARING AGENTS.

Under this heading it is only necessary to make a few observations, as several commonly employed clearing agents have been already mentioned in connection with the staining processes.

The section should be thoroughly dehydrated in absolute alcohol* before the clearing agent is applied. This rule is not followed in the case of celloidin sections, owing to the solvent action of absolute alcohol upon celloidin. Such sections are usually imperfectly dehydrated (alcohol 95–96 per cent.) and cleared by special means (mentioned below). Nikiforoff, however, states (what is the case) that celloidin sections may be thoroughly dehydrated by a mixture of absolute alcohol and chloroform, equal parts. This does not dissolve celloidin.

The following clearing agents are in common use:—

Clove-oil.
Creosote.
Origanum-oil.
Bergamot-oil.
Cedar-wood-oil.

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* Alcohol absolute enough for ordinary purposes is prepared as follows:—Calcine some cupric sulphate; when it has become white, reduce to powder. Add a quantity of this to the alcohol; the copper is allowed to remain in the bottle. If it turns blue, replace by fresh (Ranvier's plan).

Xylol.
Naphtha.
Turpentine.

Sections become brittle if allowed to remain long in clove-oil. On the other hand, it is powerful and rapid. It dissolves celloidin, and therefore is avoided in the case of celloidin sections. It causes Weigert's stain to fade, and also abstracts most anilin stains from sections, and produces alterations in the anilin colours in course of time. These disadvantages limit the use of clove-oil.

For clearing sections stained after Weigert's hæmatoxylin method xylol is generally used. It affects neither the stain nor the celloidin with which the sections are commonly infiltrated. [Origanum, bergamot, cedar, are also indifferent towards celloidin].

Sections stained by anilin dyes may be cleared with xylol, as it is an indifferent body towards these colours. As soon as the anilin-stained section comes into xylol abstraction of the stain is at once checked. The alcohol finally used for dehydration should be absolute if xylol is to be used. Sections become brittle if left long in the latter medium.

Other clearing fluids which have no effect upon anilin dyes are cedar-wood oil, bergamot oil, and turpentine.

For clearing sections imperfectly dehydrated—as a series of celloidin sections dehydrated by alcohol 96 per cent.—a mixture of xylol 3 parts, carbolic acid 1 part [Weigert], is to be recommended. In the case of anilin stains, replace the carbolic acid by anilin oil. This mixture may be kept free from water by the means recommended above for dehydrating alcohol.

For preparing objects for paraffin-infiltration cedar-wood oil is one of the best media, owing to its power of rapid penetration. Sections cut in paraffin may be placed in naphtha, xylol, or turpentine, any one of which will dissolve out the paraffin and clear the section at the same time.

The Half-Clearing Method.—Sections only partially cleared show various details of structure invisible in wholly cleared ones The half-clearing method and the principle upon which it is based are as follows:—Sections, stained or unstained, are treated with alcohol which is short of absolute (methylated spirit or alcohol 94 per cent. is suitable). The alcohol only partially dehydrates the section; certain tissue elements retain water more obstinately than others. As the clearing agent which is now applied is immiscible with water (or, at any rate, mixes very slowly with it), no clearing action (or a slow action only), is exercised upon the elements referred to. These, therefore, stand out in relief, owing to the difference between the index of refraction of their contained water and that of the clearing agent.

The *Henle-Merkel* Half-Clearing Method is as follows:—A section is placed in alcohol of about 94 per cent., in which it remains at least 10 minutes. It is then withdrawn, and the alcohol rapidly removed by filter paper or linen, until the preparation begins to look dry. Transfer to xylol in a watch-glass, and in a few moments place on slide and mount in xylol. At first only axis-cylinders are visible, but presently the ganglion-cells with their processes appear. Other tissue-elements remain invisible. The preparation keeps for about six weeks in canada-balsam.

Bevan Lewis places sections—unstained or stained in carmine—saturated with spirit, on a slide. When the spirit has nearly evaporated a drop of oil of anise is allowed to flow *over* the section, and the clearing watched under the microscope. At a certain stage a sudden starting-out in relief of cells and nerve-fibres is observed. If at this point a little balsam is dropped upon the section the appearance referred to is fixed, for some time. Instead of oil of anise, glycerine followed by mounting in glycerine jelly, may be employed.

Greppin's Method is as follows:—Sections of tissue hardened in Müller are placed for 5–10 minutes in conc. aq. sol. safranin. Wash lightly, place on slide and cover with a few drops 1–10 per cent. sol. caustic soda. Adjust cover-slip. In ½–1 hour the preparation clears up, and the medullated fibres ("even the finest of the outermost layer of the cerebral cortex"), are brought to view. The preparation keeps only 1–2 days.

IX.—MOUNTING MEDIA.

The following are commonly employed:—

Glycerine* (Price's.)
Glycerine jelly† (Rimmington's).
Farrant's solution.
Canada balsam.
Dammar varnish.

The first three are employed for mounting tissues direct from water. Sections stained with picro-carmine or osmic acid, and unstained preparations are usually mounted in these media; Golgi's sublimate sections often.

The last two are used after dehydration and clearing.

When the first three media are employed it is necessary to cement the cover-slip. With balsam and dammar this is unnecessary.

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* Instead of glycerine, levulose is often used. It preserves the colour of carmine and coal-tar stains well. Sections are brought into it out of water.

† Formulæ for making this are given in works on general histological technic.

Farrant's Solution is made as follows:—

Glycerine	equal parts.
Sol. arsenious acid (sat. by boiling)	
Water	

Mix well. Add about one-half the bulk of white gum acacia; stir well, and cover the jar. Allow to stand about three weeks, stirring daily. The gum is gradually entirely dissolved. Filter through filter paper under a bell jar (to exclude dust).

The fluid dries at the edge of the cover-glass and so fixes this slightly to the slide—an advantage over glycerine.

Canada-Balsam is prepared for use by heating it gently until it becomes hard. The hard, vitreous substance is then dissolved in benzole, chloroform, or xylol, to a suitable consistence. Filter through fine cotton wool.

It is customary to keep two balsam solutions, one made with xylol—for use with sections cleared by that reagent, the other with benzole or chloroform—for sections treated by other clearing agents. Instead of the two solvents last-named some prefer a mixture of turpentine and chloroform, equal parts.

Dammar Varnish is used, generally speaking, for the same objects as canada-balsam. It is believed by many to give a better definition of fine details. It also sets quicker. Prepare by dissolving $\frac{1}{2}$ oz. gum-dammar and $\frac{1}{2}$ oz. gum mastic in 3 ozs. benzole; filter. Or the solution may be made with xylol.

Colophonium, dissolved in benzine, or chloroform, is recommended by some, principally on the ground

that it does not become yellow with time, as is noticeable in the case of canada-balsam

Cements.

Zinc-white Cement.

Gum-dammar	8 ozs.
Oxide of zinc (finely ground) . . .	1 oz.
Benzole	8 ozs.

Dissolve the dammar in the benzole, add the zinc, and strain through muslin.

Kitton's Cement.

White lead	equal parts.
Red lead	
Powdered litharge	

Mix and grind in mortar. Rub up with turpentine, and add sufficient gold size to make a suitable mixture for working with a brush. See that no gritty particles remain.

Before applying a cement, dry the slide well up to the edge of the cover-slip. When Farrant's medium has been used, cementing should be delayed for two or three days to allow the gum to set at the edge of the cover. A ring of gold size, or thick gelatine solution, is first run round the margin of the cover. In about twenty-four hours, paint on the cement. A second ring may be applied after the first is dried.

A good plan is to employ both the cements mentioned. Apply a coating of the lead cement, and a second as soon as the first is dry. When the second

is dry, run on a layer of gold size. When this is dry, apply the zinc cement. In these manipulations a turn-table is useful.

Apáthy recommends highly a cement made as follows: Equal parts of hard paraffin (melting point = 60 degrees C.) and canada-balsam are melted together in a porcelain capsule over a moderate flame, until the whole gets a golden colour, and the odour of turpentine ceases to be appreciated. On cooling, the mass sets hard. Warm for use. Apply with a glass rod.

X.—GENERAL PLAN OF PROCEDURE IN MICROSCOPICAL EXAMINATION OF BRAIN.

Take out the brain as soon as possible after death. Remove at once a piece of suitable size—usually from the upper end of a central gyrus—and cut fresh sections on the ether-freezing microtome, according to the method described on pp. 1 and 2. Proceed subsequently as there directed, examining sections unstained and stained.

Other pieces should be placed in methylated spirit twenty-four hours, thereafter to be hardened in chrome salts (in the dark), according to Method I or II. (*See* "Methods suitable for General Purposes," pp. 24 and 25.) When hardened, wash out excess of chrome in running water for a few hours before proceeding to cut.

With some of these pieces, after-hardening in alcohols of increasing strength may be adopted (p. 25). In any case, the tissue will commonly need to be dehydrated in alcohol before cutting. The subsequent steps, now merely indicated, have been described in their proper places. The tissue is infiltrated with celloidin, or not, according to consistence and size, fixed to a cube of wood, and cut in the sliding microtome. The celloidin may be removed from the sections, if this can be done with safety. Stain

with anilin blue-black, carmine, one of the carmine combinations (alum-, borax-, lithium-carmine), alum-cochineal, or one of the combination stains (hæmatoxylin and benzo-purpurin, hæmatoxylin and eosin, anilin blue-black and picro-carmine). Wash, dehydrate, clear, mount, as directed.

Certain pieces—from which sections are to be taken for Weigert's hæmatoxylin process, or the Weigert-Pal process—are placed direct from the body in 1 per cent. sol. potass. bichrom., changed next day to 2 per cent. solution, and gradually increased to 4 per cent. solution (p. 24). Or they may be placed in Müller's fluid, to be exchanged, when the tissue is already fairly firm, for 4 per cent. sol. potass. bichrom., in which they remain till hard enough. Subsequent washing in water should be of brief duration, and may be omitted if the piece is to be in alcohol for after-hardening. Imbed, or not, in celloidin, as desirable, cut sections, and proceed according to the directions given (pp. 69 and 70). When pieces are placed immediately, without the preliminary use of spirit, in a weak chrome solution, it is advisable that they should be thin, to ensure proper permeation. A piece from a cerebral gyrus may be $\frac{1}{2}$–1 cm. thick. Bulky pieces should be incised as freely as possible.

Instead of Weigert, or Weigert-Pal, one of the other methods for medullated nerves may commend itself.

The sliding-microtome has been recommended above for cutting hardened tissues, but these may be cut also on the freezing microtome, after soaking in gum, acording to the directions given under Section-cutting ("The Freezing Method," pp. 40 and 41).

A few pieces, 1–2 ccm. (freshness of tissue is in this

case especially desirable), are placed in absolute alcohol, and treated according to Nissl's method, or Rehm's modification of it (pp. 109–111).

It is further advisable to use one of the metallic stains whenever time permits. The Golgi-Cajal method may be especially mentioned (pp. 92–95).

APPENDIX.

MUSEUM SPECIMENS.

The following are amongst the best methods of preparing brain for museum purposes.

Giacomini's Method.—Especially useful for showing exterior of brain. Dissected preparations of interior also show fairly well. If the brain is fresh it is at once placed in sat. sol. chloride of zinc. If the subject has been dead some time, inject about 600 grms. of the solution through the carotids, using slight pressure only The brain floats in the zinc solution. It should be turned frequently. After forty-eight hours remove the lepto-meninges. The organ is kept in the zinc solution 4–6 months. It tends to sink. Transfer to methylated spirit for fourteen days. Transfer to pure glycerine. The brain floats at first, but gradually sinks. It may remain 3–4 months in glycerine. On removal, wrap loosely in a cloth, and allow to dry; when dry, paint with paper-hanger's varnish. (Mastich varnish is still better). When no longer sticky the preparation may be put in the museum, covered by a glass shade, from the wooden bottom of which it is separated by a bed of cotton wool.

Blood-vessels (*e.g.*, circle of Willis, showing abnormal distribution or atheroma of vessels) may be passed through the same process, and fixed on a glass plate.

Giacomini preparations sometimes crack superficially, and also shrink with the lapse of time. They may be fairly well renovated by replacement in glycerine for some months, after removal of the varnish by brushing over with spirit.

Whitwell obtains preparations which resemble the natural brain tint more closely than the ordinary Giacomini ones, and are equal to the latter in other regards, by using—in place of zinc solution—carbolic acid 1–20. The spirit and glycerine subsequently used are slightly acidified by acetic acid. Proceed as in the original process.

In the above account of Giacomini's method it has been recommended to employ the zinc solution for a period considerably longer than that usually given. Experience shows that the prolonged use of zinc is necessary to insure durability of the preparation.

Old spirit preparations may be utilised for this method with much success. Place them in the zinc solution and proceed as described, avoiding the spirit after the zinc, as unnecessary.

Schwalbe's Method (a modification of that originally given by Fredericq).—This is more adapted for portions of brain than for the entire organ. A hemisphere, however, can be quite well treated. After washing away the blood in water and placing cotton wool in the sulci (to keep them open), the fresh brain is placed in chloride of zinc, saturated solution. Remove the pia in a day or two. After hardening (3–5 days), make any necessary sections. Transfer to spirit (about fourteen days, to further harden and dehydrate). Dry the surface, and place in oil of turpentine. A whole hemisphere remains in this about eight days, smaller

pieces a shorter time. Transfer to melted paraffin (paraffin melting at 45–50 degrees C.) Keep the oven at 60 degrees C. A whole hemisphere is kept in this 5–8 days. Preparations become rather yellow. On removal, allow the superficial paraffin to run off; cool.

The preparation appears like a paraffin model. There is but little shrinkage.

Dr. A. W. Campbell (Rainhill Asylum), employs the following paraffin method:—Place the brain, freshly removed, in sat. aq. sol. bichloride of mercury for forty-eight hours. Wash in water, transfer to methylated spirit, and leave until firm and dehydrated (3–5 weeks). Remove, dry the surface carefully with a soft cloth, and immerse in oil of turpentine for three days, at a temperature of 45 degrees C. Transfer to melted paraffin (soft variety), and keep in oven at melting point of the paraffin 4–5 days. Remove, allow to cool, and then carefully take away all paraffin from the surface. Paint with spirit varnish.

Blackburn's Method.—Harden the brain in Müller (five weeks) or Erlitzky's fluid (a shorter time), wash in water, pass through alcohols of increasing strength (for dehydration), finishing in absolute alcohol. Then transfer to a sat. sol. Japanese wax in chloroform. Here the preparation remains till the alcohol is thoroughly displaced by the chloroform; change occasionally. Transfer to melted Japanese wax, kept at a temperature near the boiling point. Here the preparation remains till thoroughly infiltrated. The time depends, of course, upon the size and density of the piece. A hemisphere, after thorough dehydration, may remain at least three days in each of the two solutions last mentioned. Infiltration complete, remove

the preparation, drain off the wax from the surface and allow to cool. When cool, varnish. The surface may be painted or lettered, if desired.

Flesch's Method.—The brain is placed in water for the removal of the blood (1–2 days); then in methylated spirit, upon a thick layer of cotton wool; turn daily. After four weeks in alcohol, transfer to weak glycerine solution (glycerine and alcohol, equal parts), two weeks; then to pure glycerine, four weeks. Each solution contains sublimate in the proportion 1 : 3,000. (Dissolve the sublimate in a little water, and add this to each solution). Allow the organ to dry. Varnish.

Lenhossék's Method.—Harden the organ in alcohol, Müller, or zinc. It must finally be placed in alcohol. The sulci may be kept opened during hardening by cotton wool. Allow the alcohol to evaporate from the surface, and then paint on everywhere, in sulci and over gyri, a solution of celloidin in alcohol and ether (equal parts), of medium thickness. The celloidin dries in ten minutes or so, and the organ is then placed in spirit, where it is kept permanently, being only taken out for demonstration. It can be exposed for about two hours without risk, after which replacement in alcohol is necessary.

FLUID PRESERVATIVE MEDIA.

(Especially for slices and portions of brain, for pons or medulla, and for growths and other lesions of the membranes).

Spirit.—Soak the tissue in a mixture of methylated spirit and water, equal parts. Change until the liquid

remains clear. A similar mixture may be permanently used.

Glycerine, to which is added some carbolic or boracic acid, or thymol.—The preparation may be in glycerine diluted with water about one half, until the liquid remains clear.

Saturated Solution of Boracic Acid (suitable for showing lesions of membranes, and for delicate tissues).—Boil an excess, allow to cool, filter through paper. Change the fluid till clear.

Grawitz's Fluid (for same purpose as last).—Common salt 150 grm., sugar 40 grm., saltpetre 20 grm., water a litre. Add 3 per cent. boracic or tartaric acid, to render acid. Change the fluid till clear.

Müller's Fluid followed by Glycerine.—Harden in Müller as usual. Transfer to glycerine. Here brain-tissue turns dark green, and on slices of brain a good differentiation of grey and white matter is obtained. The preparation remains in glycerine an indefinite time. On removal, wash lightly with water, and transfer to spirit, in which keep. [Such preparations first shown to writer by Dr. Whitwell, Menstone Asylum.]

If the preparation is put up in a glass jar, containing the fluid preservative, it may be slung by fine thread to a piece of cane (with slits for the thread) passing across at the upper part of the jar.

If dishes are used the preparation may be fixed to the bottom by plaster of Paris.

Membranes may be stretched on a slip of glass by stitching their edges round its back.

It is probably best not to seal down covers of jars containing preparations in fluid media. Fix the cover down merely with a thick mixture of wax and lard, and

apply the metal ring to the margin. The cover is then removable, and the fluid can be renewed from time to time.

Glycerine-Jelly as a Preservative Medium (for healthy and diseased sections and pieces of nerve-tissue in the fresh state, and portions of diseased cerebral meninges and blood-vessels).—This is a very suitable preservative for many purposes. The writer prepares it as follows :—Take best French gelatine (gold label) 8–10 grm., glycerine 25 ccm., sat. sol. boracic acid 75 ccm. Dissolve the gelatine, cut up, in the boracic solution by heat, add the white of an egg, and apply heat until the albumen has thoroughly separated out; add the glycerine. If the fluids are contained in a flask (closed by a loosely-fitting plug of cotton wool), and heat is applied to a basin of water in which the flask floats, but little loss by evaporation occurs. Filter through a hot water filter. The jelly should be quite clear. It will be slightly yellow. If this is thought undesirable less gelatine must be taken; the proportion given above is that used for culture-media, which remain solid throughout the year in this climate, and show no shrinkage for a very considerable period. A quantity of the jelly may be kept in stock in a sterilised flask, plugged by cotton wool.

The preparations are put up in glass vessels. Petri's, Esmarch's, or other dishes are suitable. The glass or earthenware dishes mentioned in the list of apparatus may be used for large preparations. Pour some of the melted jelly into the vessel, let it set; arrange the preparation on its surface, and cover with more jelly. Air bells are removed by a camel hair brush. If a fresh section of brain is to be mounted direct from

the freezing microtome it may be floated off the knife on to sat. sol. boracic acid; the glass preparation vessel, half-filled with set gelatine, is passed into the fluid beneath the section, and the latter arranged with a camel hair brush upon the jelly. Vessel and section are then withdrawn, excess of fluid is run off, and melted jelly (not too hot) poured in up to the brim, care being taken to prevent floating of the section.

The covers of these vessels may be left unsealed, fresh jelly being added from time to time; or they may be sealed. The writer has sealed specimens rather over two years old in which no appreciable shrinking of jelly has occurred. Preparations may be tried in both ways. For sealing, Kitton's cement may be employed in the first place. Put on two or three coats, allowing each to dry before applying the next. Then apply a layer of gold size, finishing with a coating of zinc-white cement. [*See* "Cements" for composition of above mixtures]. Finally paint over with black bicycle varnish. The bottom and sides of the vessel may be painted with the same; thus a good back ground is afforded.

Lesions of the lepto-meninges—such as opacity, hæmorrhagic effusion, and adhesions of cortex cerebri to pia—may be well shown by mounting in a thin layer of glycerine-jelly between two squares of window glass. Air must be carefully excluded (a troublesome matter). During the preparation the plates should lie on a levelling tripod. One plate is first covered with jelly and the membrane arranged on this; more jelly is poured over the tissue, and the second plate then brought down on the preparation, like a cover-slip over a section. Fill in jelly up to the extreme edge of the

plates. Cement the edges as already described. The procedure needs practice.

These fresh jelly preparations keep well. At the West Riding Asylum are some two years old, apparently unaltered, except as regards blood tint; this has undoubtedly faded. The blood tint cannot be properly preserved by any known process. But in certain of these jelly preparations of lepto-meninges the blood tint is better preserved than in preparations by any other method which the writer is aquainted with.

Atheroma, and obstruction of blood-vessels, abnormal arrangement of the vessels at the base of the brain, are well shown in jelly preparations.

For mounting thick slices of hardened brain glycerine-jelly is very suitable. The writer recommends Hamilton's method, which is as follows:—The brain is hardened in Müller which is subsequently soaked out by steeping the organ in solution of sugar of greater specific gravity than the Müller, and changing frequently until there is no further discolouration. A slice of the brain is placed for at least a week in Hamilton's freezing fluid "B." [*See* "Section Cutting; Freezing Method."] Freeze and plane down to the necessary level in his large microtome. The slice is then stained (not too deeply) in a mixture containing ammonia-carmine 1 part, freezing fluid "A" 8 parts. After washing lightly in water, transfer to a sol. of glycerine 1 part, sat. sol. boracic acid 2 parts. Here the slice lies for 3–4 days. It is now ready for mounting. Prepare a cell by fastening four strips of plate glass, 1 inch in breadth, by zinc cement (*see* "Cements"), to a piece of window glass. The cell is quadrilateral. Cement is run in between the ends of the strips, so

that the cell wall is complete. The cell is half-filled with glycerine-jelly [the formula given before may be adopted]. Remove air bells with a fine brush. Allow the boracic solution to run off the slice of brain, and then slip the latter gradually into the cell. Fill up with jelly as nearly to the top as possible. Use a levelling tripod. Allow to solidify overnight. Then run some melted jelly over the solid, and slip on a cover of window glass, large enough to extend half-way over the wall of the cell. Leave the preparation to cool, and then clean up the edges. Cement as follows:— A strip of clay 1 inch broad is placed round the preparation, one-half inch from the edge. Into the trough so formed pour melted asphalte, composed of pitch, sand, and Archangel tar (1 part tar to 8 parts pitch). The mixture is used at the lowest possible temperature. The edge of the cover-glass is covered with it also, for a breadth of one-quarter inch. When the asphalte is hard and cold, remove the clay walls, and clean the preparation.

If desired to show both sides of the tissue each should be planed down in the freezer; otherwise the back of the preparation may be painted black. Cover the asphalte and edges with paper.

Hamilton's Gelatine-Potash Method for large naked-eye sections of brain, should be included amongst methods of preparation. The process is lengthy, and for this reason is omitted here. [*See Brain*, July, 1883, and *Text-Book of Pathology*, by D. J. Hamilton, pp. 48–52].

Lesions visible to the naked-eye in microscopical sections are shown very well by mounting the sections, after staining, dehydrating, and clearing as usual, in

balsam, between two strips of window glass. The sections are arranged in a row; two parallel rows may be made—the one showing the diseased, the other the corresponding healthy sections. Sclerosis of gyri, lesions of the internal capsules, hemi-atrophy of pons and medulla, tract degenerations of the cord, &c., may be shown in this way. Sections may, of course, be examined under the low-power also. Take a strip of window glass, about 3 inches broad, and of any desired length. Remove the sections from the clearing fluid, and arrange them on the glass. Remove excess of clearing fluid, and cover each section well with balsam. The balsam is now allowed to dry to a sufficient extent to prevent the sections from moving during the subsequent procedure (some hours). The glass slip is next covered with balsam to within a short distance of its edge. Now cover with a second slip precisely like the first; make contact between the slips, through the balsam, first at one end, then, lowering the upper slip gradually, along the whole length, so that the balsam is spread out evenly between the slips. With a little practice air-bubbles can be entirely excluded, and the space between the slips completely filled with balsam. They are practically in contact when the operation is complete. Balsam exudes freely at the edges, so that it is advisable that the slips should rest upon two glass rods to prevent adhesion to the table. Leave the balsam to dry under a bell-jar. When dry enough, remove what has exuded by means of a cloth dipped in benzole. When the balsam has thoroughly set, clean up the slips, and place in the museum upon suitable glass or wooden rests.

Plaster Casts of Brain* may be made as follows (the method has been some time in use at the West Riding Asylum; it was first employed, the writer believes, by Bevan Lewis):—Place the brain in a suitable position in a basin, and pour over the surface melted paraffin (hard quality), sufficiently warm to ensure penetration into the sulci. It must not be allowed to approach too closely the setting point; on the other hand, too great heat must, of course, be avoided. The pouring is done deliberately. Finally the brain is completely hidden by solid paraffin, which also fills in the space between it and the basin wall. This procedure occupies 4–5 minutes. As soon as the paraffin is well set, cut through the portion surrounding the brain down to the bottom of the basin in a complete ring round the organ. Place the basin and contents in cold water—the paraffin sets hard. Now remove from the water, and turn out the brain covered by its paraffin cap, using a strong scalpel for the purpose. By a little shaking the cap can be separated from the brain; from its inner surface numerous ridges and processes are seen projecting; these correspond to sulci. A mould of the brain is thus obtained. Fill it with good plaster of Paris, made up to the usual consistence of a cast with water. When the plaster

* Instead of plaster of Paris the writer has lately used the glue and treacle mixture known as "printer's roller composition," and obtained at the printer's. This makes a capital cast. The material is poured (at a suitable temperature) into the paraffin mould, and allowed to set. It can be shaken out of the mould readily. If desired, traction can be made upon a piece of tape, previously arranged in the glue whilst still liquid. By mixing some whiting with the glue a tint nearly approaching that of the brain can be obtained.

has thoroughly set, place the whole in water in a saucepan, and heat. The paraffin melts away, leaving a cast of the brain. The convolutions and sulci are well mapped out, the under surface is quite flat. Any little holes—sometimes such are seen: 1–2 mm. diameter—may be filled up with plaster. Expose the cast to a gentle heat, to dry it. Subsequently it may be painted of the same tint as a Giacomini preparation.

Abnormal disposition of gyri, difference in size of hemispheres, local atrophy and depressions, gaping sulci, and other lesions may be represented with accuracy by this method.

Paraffin Casts of Interior of Skull.—Proceed as follows:—Saw off the skull-cap. Remove the brain and its membranes. Fill up the basal foramina with clay. Saw a wedge-shaped piece of bone out of the occipital region, but leave it *in situ*. Raise the body into a sitting posture by rope and pulley. Fill the base with melted (hard) paraffin. Throw a piece of strong tape transversely across the base so that some projects a foot or so on either side. Trephine a hole in the vertex of the skull and replace the skull-cap. Fill up the space between skull-cap and base with clay, and surround this part with a bandage spread with clay. Pour in more paraffin through the trephine hole until the cranial cavity is full. When the paraffin has set, place the body horizontal, remove the skull-cap, and the wedge-shaped piece of bone from the occipital region, and extract the paraffin cast. This is accomplished by a combination of pressure on the cast from behind, traction on the tape, and tapping the base of the skull on the cut surface of the bone and laterally. After removal, cut off the projecting tape. [This is

much the same process as that recommended by B. Lewis for the estimation of cranial capacity.]

A cast of the skull-cap alone (as for the purpose of showing a-symmetry), or of the base of the skull, may be taken, in plaster or paraffin—preferably the latter.

If skull-caps are to be preserved for the purpose of showing anomalies or lesions, the following method of preparing the bone may be recommended:—Soak for several days in strong solution of caustic potash. The soft parts can now be easily scraped off. Wash next in water for several days; dry, and varnish. Place on a suitable support.

In addition to the methods described and recommended above mention may be made of the following:—

Stieda's Process. — This is a modification of Giacomini's method.—Place the brain in chloride of zinc, sat. aq. sol.; leave here twenty-four hours. The pia is now removed. Transfer to alcohol, 96 per cent.; change every 5–6 days. The brain is sufficiently hard in 2–3 weeks. Transfer to turpentine, 2–4 weeks. This penetrates the quicker the better the brain has been dehydrated; at least two weeks needed. The organ becomes again rather soft, but "transparent," and also acquires a brownish tint. Place in varnish (so-called "drying-oil") for two weeks. Remove, and allow to dry thoroughly in the air. Experience shows that much more shrinkage takes place by this method than by Giacomini's, and that a cheesy dryness of portions of the surface quickly occurs, even when much more time than recommended is spent over the process. By stopping short of the varnish stage and leaving the

brain permanently in the turpentine, the writer finds that good museum preparations can be obtained.

Stieda suggests that thick slices of chrome-hardened brain should be treated, for purposes of demonstration, with turpentine and varnish, as in his method.

Thoma's Process for Preserving the Natural Tint of Tissues.—Pieces not thicker than a finger are suspended in one of the following fluids, which may apparently be used indifferently:—

	A.	B.
Crystals of sulphate of soda .	100 grm.	60 grm.
Chloride of sodium	100 „	100 „
Chloride of potassium . . .	100 „	30 „
Nitrate of potash	10 „	10 „
Water to make	1 litre	1 litre.

After 18-24 hours, brush off any superficial blood, and place in spirit, which should be changed once or twice. Keep in spirit. A preparation is obtained which shows at least a considerable amount of the original colour. After some months the red tint of hæmoglobin changes to the brownish-red of methæmoglobin. Notwithstanding, preparations over four years old are said to afford better demonstration—objects than can be made by the ordinary methods (preservation in spirit and other fluid media). The writer has tried Thoma's method with cerebal tissues and membranes, but without any advantage. The original results appear to have been obtained with spleen, liver, and kidney.

Thoma states that the microscopical structure of organs is well preserved by his fluid, and recommends, in cases in which it is desired to preserve the red corpuscles of blood in microscopical preparations, that

Müller's fluid and other chrome solutions should be replaced by one of the following hardening fluids, which interfere less with subsequent staining than do the chrome solutions:—

	C.	D.
Crystals of sulphate of soda	60 grm.	50 grm.
Sodium chloride . .	60 „	30 „
Potassium chloride . .	20 „	10 „
Nitrate of potash . .	10 „	10 „
Water to	1 litre	1 litre.

To Clean New Cover-Slips and Slides. — Place them in strong nitric acid for an hour or so. Wash very thoroughly in water, to remove acid. Then wash in methylated spirit, two changes. Wrap in a clean cloth, and leave in incubator (37 degrees C.) a few hours. On removal the slides and covers are clean. Rub over with a clean cloth immediately before use, or —a better plan, especially for cover-slips—place upon a wooden board covered with wash-leather, and polish with a strip of the same. There is then no risk of fracture of cover-slips. Or the slides and covers may be kept in spirit until required.

To Clean Used Covers and Slides.—Remove the covers by soaking them and the slides in used xylol. Scrape the slides free from the mounting medium, and transfer the several glasses to used spirit, thence to a mixture of spirit and hydrochloric acid (strong), equal parts. In a few days place in following solution:— Water, 2,000 parts; pot. bichrom., 200 parts; strong sulphuric acid, 200 parts. Here the covers and slides remain some hours. Wash well with water, and then with changes of spirit. Dry, or keep in spirit.

LABORATORY EQUIPMENT.

Supposing the laboratory to consist of four rooms, one in which the hardening of tissues is carried out, a second—the antopsy-room, a third, serving as the histological work-room, and a fourth, the museum—a convenient distribution of the apparatus will be necessary. The most important apparatus and instruments required, together with general indications for their convenient distribution, are given below. [There is no intention of entering here into the structural details of the laboratory.]

In the Hardening Room.

This room should be kept cool, and should contain cupboards, so that hardening may be carried out in the dark.

Several Winchester quart bottles of dark blue glass,* for the hardening solutions.

Several large (two-pint), wide-mouthed, well stopped bottles for chrome salts, or other solid reagent.

Some smaller sizes of the same, both stoppered and corked.

Glass vessels, with glass cover and metal rim, for holding hardening brain.

Some smaller sizes of same, for portions of brain, cerebellum, medulla, and the like small organs. Marmalade pots are useful for this purpose.

Earthenware jars with earthenware lid and iron clamp, are also useful for holding the hardening brain.

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* Either this tinted glass should be used or the bottles kept in the dark when containing solutions of the chrome salts.

Cord jars, 21 inches long and $2\frac{1}{2}$ inches in diameter, with hook to cover.

Glass flasks of various sizes.

Nest of beakers.

Glass funnels, different sizes.

Filter stand with ring supports—different sized rings.

Hot-water filter.

Filter paper, different sizes.

Syphon bottle (holding several litres), for mercurial and carbolic disinfecting solutions, with tube and pinch-cock.

A large glass vessel for the zinc solution used for hardening brain in Giacomini's process—about 12 inches diameter and 10 inches high. A glass shade of suitable size, fixed upside down to a wooden support by plaster of Paris, answers well.

One or two large Wolff bottles.

Squares of glass, various sizes, for covering vessels.

Indiarubber and glass tubing, various sizes.

Screw pinch-cocks.

Indiarubber and ordinary corks.

Nest of cork borers.

Distilling apparatus—one for water and another for spirit—of copper.

Bunsen burners, a few. Also the rosette, and any other suitable burners of Fletcher, Warrington.

Foot-bellows, attached by rubber tubing to blow-pipe [Fletcher].

An incubator, with Reichert or other regulator, and thermometer, and suitable jet burners with mica cylinders.

Paraffin oven, with regulator, thermometer, and burner.

A few copper dishes, with handles, for holding melted paraffin with contained object.

Porcelain crucibles, different sizes.

Tin saucepan, for melting paraffin and wax.

Tripods, different sizes.

Ice safe.

Water bath.

Zinc saucepan, and some suitable copper utensil, for boiling water in, cleaning slides in, &c.

Porcelain evaporating dishes, different sizes.

Stone mortar and pestle, two or three sizes.

Stone jar with glass tap, two; very convenient for holding distilled water and spirit, near the work table.

Wire and gauze.

Specific gravity jars and bulbs (Sankey's method).

Mercury manometer.

Brass syringe, with cannulæ of different sizes, and clips for blood-vessels.

Glass syringe.

Levelling table, with spirit-level.

File, hammer, chisel, and other tools.

In the Autopsy Room.

The ordinary *post-mortem* apparatus and instruments (use gramme weights).

Apparatus to facilitate sawing of the skull. This consists of a steel cinglet which is screwed on to the skull just above the level at which it is proposed to saw the latter. A steel arch passes vertically from front to back of the cinglet, over the head, and serves as a handle. The bone is sawn at the lower margin of the cinglet.

Apparatus for raising the body into a sitting position,

in order that a cast of the interior of the skull may be taken. This consists of leather straps which can be buckled round the head and beneath the chin, and rings attached thereto; also a suitable arrangement of ropes, with hooks, and a pulley.

Tables comparing the metric and English systems, and the Centrigrade and Fahrenheit scales. These may be hung in a prominent position.

A table showing the average weights of organs in grammes and ounces avoid.

Whitwell's brain-slates for recording lesions [Danielsson & Co.]. This method is a great improvement on the ordinary one (diagrams on gum-backed paper). Instructions issued with the slates.

Thermometer, for recording temperature of room.

Millimetre rule (Zeiss supplies), for measuring depth of cortex, &c.

Steel tape measure (centimetre and inch).

Ether-freezing microtome. The autopsy—or the hardening—room is an appropriate place for this, as the temperature is suitable for ether-freezing. Bevan Lewis's instrument may be used (Gardner, Forrest Road, Edinburgh), or the ether-freezing apparatus of Cathcart (A. Fraser, 7, Lothian Street, Edinburgh), or Jung (Heidelberg). Rutherford's ice-freezing instrument has also been adapted for ether (Gardner, Edinburgh). The materials required for Lewis's fresh method should be at hand, namely: bottle of ether, spray apparatus, a couple of circular glass vessels for water, about 10 inches diameter and 4 inches high (as used in Koch's plate culture method), aq. sol. anilin blue-black, $\frac{1}{4}$ per cent.; osmic acid, $\frac{1}{4}$ per cent. sol. (in green glass or covered bottle), two pipettes with indiarubber balls attached—

for dropping these solutions on the sections, a camel hair brush, a couple of mounted needles, slides. Also a covered wooden case, with rests, upon which the slides with their sections can lie in a slanting position, whilst the latter are drying.

In the Histological Work Room.

A microscope, from one of the well-known firms. It should have two oculars, weak and strong, at least three lenses (say one-half and one-sixth inch, and one-twelfth oil immersion), an Abbe condenser with iris diaphragm, and a nose-piece for three lenses. With apochromatic objectives compensation-oculars are employed.

For photo-micrography, apochromatic objectives, with achromatic condenser, and projection eye-pieces.

It is convenient to have as well a cheaper, simpler instrument.

A camera for photo-micrography (long extension, 20–30 inches). Heat-filter and oxygen-cylinder. The camera should stand on a strong, steady table.

Glass shades for the microscopes.

Microtomes. A sliding and a well instrument. A special instrument for paraffin objects is not necessary, but is useful. The instruments recommended were mentioned under "Section-Cutting."

Knives for cutting frozen and wax sections. Two kinds sufficient. The one, 6 inches by $\frac{3}{4}$ inch, fixed in a handle; the other, a clasp-knife, 5 inches by 1 inch. (Young and Gardner, Edinburgh.) They should be hollowed on both surfaces, more on that uppermost in cutting; back and edge on the same plane.

Wash-leather cases for knives.

Hone, of Turkish stone.

Leather-strop, of bootmakers' "uppers." Polish the surface with a thick mixture of "Tripoli" and olive-oil.

Calves-skin strop, for finishing.

Balance and weights.

Pair of scales.

Microscopic lamp (Swift or Baker, London).

Copper solution to be placed between light and microscope (contained in flask holding about 550 ccm.) Formula as follows :—

Water	10,000 ccm.
Liq. ammon. fort.	100 ccm.
Ammonio-sulphate of copper sol.	20 ccm.

The last-mentioned composed as follows :—Copper-sulphate, 50 grm. ; liq. ammon. fort., 60 ccm. ; water, 200 ccm.

Or the lamp may have a blue glass cylinder.

Camera lucida. (Abbe, Nachet, Oberhäuser).

Stage micrometer.

Eye-piece micrometer.

Bull's eye condenser.

Dissecting lens.

Hand lens.

Pocket lens.

Edinger's projection apparatus (of Kanthack, Golden Square). Useful for drawing purposes, but by no means necessary.

Glass bottles for spirit and distilled water. Besides the opening above these have one below, fitted with a glass tap.

Graduated glass measures—ounce, minim, litre, cubic centim.

Graduated pipettes and burettes.

Wash bottles, one or two.

Test tubes, with rack, tube brushes, and wooden tube-holder.

Dessicator.

Air pump, with glass chamber.

Plate-glass cover for work-table. The surface of the table should be painted black, with the exception of a white band about 5 inches broad, running from side to side, a few inches from the anterior edge. Over the whole is the plate-glass cover.

Stoppered glass bottles for acids.

Same, about 6 oz. capacity, for stains, alcohol, and clearing oils.

Green glass bottles, two or three.

Phials, provided with indiarubber pipettes, for stains.

A few glass tubes with indiarubber balls fixed at one end [Sumner, Chemist, Lord Street, Liverpool]. Useful for taking up stains and fluids in quantity.

Porcelain capsules and cups, various sizes.

Glass mortar and pestle.

Some small porcelain developing dishes.

Spirit lamps.

Balsam bottles.

Esmarch or Petri dishes (Lautenschläger), useful for holding sections in spirit, &c.

Cupped glass blocks with glass cover (same maker), for staining sections in.

Watch-glasses, various sizes.

Sildes, some broad, as well as ordinary ones.

Well-slides, one or two.

Small bell-jars, for covering exposed fluids, &c.

A few forceps, ordinary dissecting, and fine.

Scissors—ordinary, and fine; straight, and curved on the flat.

Scalpels.

Razor.

Needles on handles, a few. Glass ones also.

Copper lifters (spatulæ). Glass or platinum one also.

Glass-rods, various sizes.

Camel-hair brushes.

Cover-slips, Nos. 1 and 2. Quadrilateral, of various sizes, as well as round.

Apparatus for testing thickness of covers (convenient).

Turn-table.

Labels.

Cabinet, and cardboard cases for specimens.

Centigrade thermometer.

Soft cloths, wash-leather.

In the Museum.

Round glass jars, with ground top, glass cover, and metal rim for cover, various sizes. For preparations in spirit or other fluid.

Flat (oval) glass jars, about 3 inches deep, with ground top, glass cover, and metal rim, various sizes. For suspending flat preparations in fluid media.

Square glass and earthenware dishes, 3–4 inches high, and of various sizes, with plate-glass cover. For slices

of brain, stretched membranes, and other flat preparations in fluid media and glycerine-jelly.

Petri's, Esmarch's, and other glass dishes, of various sizes, with grooved glass cover. For glycerine-jelly preparations. [Obtainable from Lautenschläger.]

Strips of window glass, 3 inches broad and of various lengths. For balsam-mounted sections.

Squares of window glass, various sizes, for glycerine-jelly preparations.

Glass shades, with grooved wooden bottom, for dry preparations.

The following firms, amongst others, supply the glass shades and various forms of glass vessels necessary for museum preparations.

F. and M. Lautenschläger, Oranienburger Strasse, 54, Berlin, N.

J. Ford and Co., Holyrood Glass Works, Edinburgh.

Pilkington, Brothers, Glass Works, St. Helen's, Lancashire.

The various reagents employed in the processes of hardening brain, in the preparation of brain by the teasing and fresh methods, in the preparation of hardened brain for cutting, in staining, dehydration, clearing, and mounting of sections have been mentioned in the description of these processes. It is unnecessary to give a list of them here. The most commonly used reagents should be kept in stock solutions.

The following have not been directly mentioned, but should be kept in stock with other material; the principal acids (sulphuric, nitric, acetic, etc.); sticks of soda and potash; vaseline and lard.

In addition to the rooms mentioned, a dark room for photographic purposes should be provided, with the

necessary apparatus, and if possible a photographic studio. Nothing will be said here about photographic apparatus, except that the camera should be capable of fixation in either horizontal or vertical position. The latter is required, for example, in photographing fresh brain. The organ is placed in water in a basin painted black; the basin stands on the floor. By immersing the brain in water the surface glistening is obviated.

SCHEME

FOR THE

EXAMINATION OF BRAIN AND COVERINGS.

Examination, — hours p.m.
Temp. Room.
Atmosphere, Humidity of { more. / less. / usual.

Scalp, Anomalies or Lesions of.

Skull-Cap { Sawn at fixed level, *e.g.*, 3 cm. above root of nose.

Thickened or thinned.
If alteration, gen., local.
Translucency, degree of.
Diploë at cut surface.
Amount.
Colour (vascularity).
Meshes open or filled up (incr. density).
Hard or soft.
If alteration, gen., local.
Pacchionian indentations.
Size.
Depth.
More or less numerous.

Skull-Cap—*continued.*

Bosses or Spicules from Inner Table.
New Layering of „ „
Bosses from Outer Table.
Colour (vascularity).
Symmetry.
Weight.
Caries of bone.
Any lesion not coming under above.
Measurements.*
(*a*) Diameters.
Antero-posterior.
Transverse.
(Others if time permits.)
(*b*) Circumferences.
Antero-posterior.
Transverse.
Horizontal.

Base of Skull.

Any lesion of, esp. caries.
Symmetry.
State of palate.

Membranes.

(*a*) *Dura Mater and processes of.*
Adherent to skull-cap.
Extent of adhesion.
Site „ „
Degree „ „
Tense { normally. / unduly.

* According to directions given by Topinard ("Anthropology").

Membranes—*continued.*

(*a*) *Dura Mater and processes of*—*continued.*

Flaccid (wrinkled).
Thickened or thinned.
If alteration, gen., local.
Colour (vascularity).
Any local congestion.
Adherent to arachnoid.
Undue amt. subdural fluid.
Quality of „ „
Semi-gelatinous, lymph-flakes.
Cysts.
Blood-effusion or new formation on inner surface, N.E. characters of.
Bony deposits in.
Sinuses of.
Capacity.
Contents.
Lining membrane.
Undue adhesion to base of skull.
Any lesion not coming under above.

(*b*) *Arachnoid* (at parts distinct from pia).

Undue granulation of outer surface.
State of Pacchionians.
Undue opacity (esp. white spots and patches indicative of thickening).
Site of.
Degree of.
Swollen, gelatinous (site).
Blood effusion in connec. with (site).
Sub-arachnoid fluid; quantity; any notable alteration in quality.
Any lesion not coming under above.

Membranes—*continued.*

(*c*) *Lepto-meninges.*

Undue opacity.
Site of.
Degree of.
Normal tenuity, or
Swollen, gelatinous.
Gen., local.
Vascularity ; excessive, or pallor.
Gen., local.
Blood effusion ; site.
Adhesion to cortex.
Extent, site of adhesion.
Strength of „
If no adhesion, how does pia strip from all parts—normally or in sheets.
Any adhesion between hemispheres.
Any lesion not coming under above.

Cerebrum.

Size.
Average.
Small.
Large.
Diminution in size of individual lobes.
Consistence.
(*a*) Indications of gen. diminution of.
(i) On superficial inspection.
Collapsed state of hemispheres.
Undue separation of „
Laceration of corpus callosum.
Gaping sulci, &c.
(ii) On Palpation.

Cerebrum—*continued.*

(*b*) Firmness of C. as a whole, average, or increased [degree of increase].

Undue surface-greasiness.

Grey Matter and Gyri.

Diminished consistence (flabby, yielding too readily to pressure, &c.).

General.

Local; old, recent (tint).

Firmness, average.

Sclerotic changes, with site.

Arrangement main gyri.

Degree of convolution.

Atrophy of gyri.

General (state of sulci).

Local (convolutions sunken below gen. level anywhere).

Colour (vascularity), on surface and section; any patchiness of colour.

Blood extravasation; site.

Erosion from stripping pia; site.

Cheesy, chalky nodules, or other focal lesions: seat.

White Matter.

Consistence; average, possibly increased.

Diminished, gen., local { tested by touch, water-stream, section; does subst. cling to knife?

Colour (vascularity).

State if local alterations.

Vessels coarse, bristly.

Blood-extrav.; seat.

Cerebrum—*continued.*

White Matter—continued.

Oedema (slighter degrees shown by brilliance of exposed surface).
Undue porosity (état criblé)
(indicative of atrophy).
Sclerosis ; seat.
Scars, cysts, nodules, or other focal lesions ; seat.
Any recent softening about these.
Other lesions not coming under above.

Basal Ganglia and Capsules.

Size (of ganglia); any shrinkage laterally (atrophy).
Consistence.
If softening, seat.
„ sclerosis, „
Colour (vascularity).
Blood-extrav. ; seat.
Scars, cysts, nodules, or other focal lesions ; seat.
État criblé (of ganglia).
Other lesions not coming under above.

Ventricles (lateral and third).

Dilated.
Excess of fluid.
Appearance of fluid removed by pipette.
(More precise examination in special cases if desirable.)
State of ependyma.
Granular, thickened, macerated.
Anomalies of cornua.
Consistence of fornix, white and grey commissures, septum lucidum.

Cerebrum—*continued.*

Ventricles (lateral and third)—*continued.*

Abnormalities of choroid plexuses and velum interpositum.

Congestion, exudation in, cysts, &c.

Other lesions not coming under above.

Corpora Quadrigemina, state of.

Pons and Medulla.

Size.

If diminution, gen., local (incl. unilateral).

Consistence.

If diminution, gen., local.

Colour (vascularity).

Blood-extrav.; seat.

Ependyma of fourth ventricle.

Does pia strip normally?

Other lesions not coming under above.

Base of Cerebrum.

Main vessels at base.

Relative size of corresponding ones.

Arrangement.

Atheroma.

Occlusion.

Cerebral peduncles.

„ nerve-roots; optic tracts.

Pitnitary body.

Other lesions not coming under above.

Cerebellum.

Size.

If diminution, gen., local (incl. unilateral).

Cerebellum—*continued.*

Consistence.
Normal.
Softening; gen., local.
Sclerosis; seat.
Colour (vascularity) of grey and white matter.
Blood-extrav.; seat.
Corpora dentata.
Does pia strip normally?
Other lesions (as growth) not coming under above.

*Weights.**
Whole brain.
Right hemisphere.
Left „
Pons, medulla, and Corp. Quadrigemina together.
Cerebellum.
Amount of fluid collected.

* And of individual lobes if desired.

ADDENDUM TO "SPECIAL STAINING METHODS."

Rosin has lately (*Neurolog. Centralbl, December* 1*st*, 1893) described a method of staining the nervous system (central and peripheral), for which he claims the following advantages:— (1) the various tissue constituents are well differentiated; (2) structures hitherto unrecognised are shown; (3) the necessary procedure is easy and rapid. The stain employed is a modification of the Biondi-Ehrlich triple stain (Säure-fuchsin, Methyl-orange, Methyl-green), of which the two first components are acids, the last is a base. The mixture has an approximately neutral reaction. From this the various tissue constituents select either acid or basic component; in some instances, again, they react to the entire neutral mixture, in accordance with their chemical affinities. The tissue may therefore be said to contain acidophile, basophile, and neutrophile substances, and thereby various colour-reactions are entailed; thus by the use of this mixture a differentiation of tissue-elements is afforded.

In staining, a distinction is made between celloidin

sections and others. For the former, a special staining fluid (made, however, from that ordinarily employed), is used. The stain for sections in general is as follows:—

A	The triple stain referred to (from Grübler)	0·4
	Distilled water	100
	Aq. sol. Säure-fuchsin . (0·5 per cent.)	7

In this sections remain five minutes. For celloidin sections use the following:—

B	Sol. A	4 parts.
	Aq. sol. Säure-fuchsin . (0·5 per cent.)	1 part.

In which the sections remain only one minute. [Both stains may be obtained ready-made from Koenig, Berlin, N.W., Dorotheen Strasse, 35]. Subsequent treatment of sections is the same whether Sol. A or B has been used. Wash in distilled water (two quantities). As soon as the formation of large clouds of colour ceases (1–2 minutes—the amount of extraction must be carefully controlled), remove sections to a solution of acetic acid 1 : 2,000 (1 drop glacial acetic acid to 100 water); here they remain five to (at most) ten seconds, with the object of fixing the red colouring matter, which is otherwise too easily extracted. Wash again in distilled water, one minute, to remove acetic acid. Transfer sections to absolute alcohol on a spatula; here they remain as long as violet colour is extracted. When no more colour, or at most only

a small amount of blue-green colour escapes (2–3 minutes), remove sections to xylol. Do not leave long in xylol. Mount in xylol-balsam.

The sections are obtained from tissues hardened in chrome and in alcohol.

Examination gives the following information :—

I. Pure acidophile tissues.

(*a.*) Purple.
- i. Connective-tissue.
- ii. Walls of blood-vessels.
- iii. Sclerosed glia.

(*b.*) Orange.
- i. Red blood corpuscles.
- ii. Medullary sheaths (only in chrome preparations).

In very old chrome-tissues the myelin is of a greenish tint.

II. Tissues still acidophile, but reacting with a rather different colour to the above (a tinge of violet).
- i. Axis-cylinders.
- ii. Protoplasm of all cells (glia-cells, chrome-containing ganglion-cells, white blood corpuscles).
- iii. Neuroglia.
- iv. Nucleoli (glia-cells and chrome–containing ganglion-cells).
- v. Nuclei of chrome–containing ganglion-cells in anterior cornua of spinal cord, and of similar large cells in cortex cerebri, also those of Purkinje's cells.

III. Neutrophile tissues (violet).

i. Nuclei of certain small chrome–containing ganglion-cells in Clark's columns, the substance of Rolando, olives, cortex cerebri and cerebelli).

ii. In alcohol-hardened tissues the nuclei of group II, v.

IV. Basophile tissues (blue-green).

All nuclei of glia, blood-vessels, white blood corpuscles, and connective-tissue ; not, however, those of ganglion-cells.

In addition, this method of staining has the following advantages :—

(1.) By reason of the alteration of colour degenerations show especially well.

(2.) Extravasations of blood are well shown.

(3.) Newly-formed blood-vessels are rendered prominent by the purple staining of the vessel-wall.

(4.) Nuclear increase is well brought out by the blue-green colour of the nuclei.

(5.) The structure of cell-body and nucleus also well shown (in alcohol preparations).

(6.) Clear differentiation between ganglion- and glia-cells is afforded. On the other hand, no colour distinction can be made between axis-cylinders and glia-fibres.

(7.) Exudates (as in central canal of cord) unequivocally shown by the red staining of the albumen.

For further details (concerning the structure of ganglion-cells, and concerning certain structures hitherto unrecognised, according to Rosin), *see* the original paper.

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